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Abstracted by Eben J. Carey, author. Marquette University
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Studies in the dynamics of histogenesis. Intermittent traction
and contraction of differential growth, as a
stimulus to myogenesis.

XI. The dynamics of the pectoralis major muscle tendon.

The origin and architecture of the pectoralis major muscle is intelligible only in relation to skeleton growth. At the beginning of the rotation of the fore limb there is no overlapping of the tendon of the pectoralis muscle. The rhythmic accelerated growth of the clavicle, sternum, vertebrae, ribs, and humerus draw out in intermittent traction the pectoralis major muscle along the successive resultants of the parallelogram of forces. The clavicle grows laterad, but the sternum cephalad, and the resultant is cephalolaterad from below, but caudolaterad from above as the limb is successively rotated ventrally. The overlapping of the pectoralis major tendon is the later resultant of the abduction and extension of the humerus caused by the synergists of the pectoralis major muscle.

STUDIES IN THE DYNAMICS OF HISTOGENESIS. INTERMITTENT TRACTION AND CONTRACTION OF DIFFERENTIAL GROWTH, AS A STIMULUS TO MYOGENESIS

XI. THE DYNAMICS OF THE PECTORALIS MAJOR TENDON

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SIX FIGURES

INTRODUCTION

Evidence was presented by the writer ('19, '20, '21) that tension of differential growth was the stimulating factor initiating the genesis of muscular tissue. The irritable primordial protoplasm responds to the initial adequate tension by contraction. The intermittent induction of the tractional stimulus is due to three factors, all of which are extrinsic to the zone of cells destined to be repeatedly stretched in the formation of muscle. These factors are: first, the dominant, epithelial growth in the tongue and intestine; second, the accumulation of fluid in the heart and bladder; third, the accelerated linear growth of the skeleton.

Differential growth connotes a dominant and a subdominant region. The former draws out the cells of the latter in tension. As regards the skeletal muscles, the subdominant area or region of retarded growth is the mesenchymal tissue that forms the muscles, the dominant zone or area of accelerated, linear growth, the skeletal elements. Muscular tissue is a sensitive indicator or tensiometer recording the degree of intermittent tension or work to which it is subjected by the extrinsic, dominating zone.

If these statements are facts and not fancy, then the origin and architecture of every type of muscle in the body, namely,

smooth, cardiac, and skeletal, may be defined in terms of intermittent tension. The genesis and growth of muscular tissues is a quantitative property of its work or exercise. This is due initially to the interaction of the intermittent tractional stimulus and the irritable protoplasm responding by contraction in areas of unequal growth.

DATA ON THE DYNAMICS OF THE PECTORALIS MAJOR MUSCLE

With these principles clearly in mind, the interpretation of figures 1 to 6 is relatively simple. These represent in diagram the later development of the pectoralis major muscle during that critical period of growth, the rotation of the fore-limb. The two zones of accelerated growth for our immediate attention are the sternal and clavicular areas. The sternum exerts its dominant growth activity in a longitudinal direction, whereas the clavicle is growing at an accelerated rate in a lateral direction. The resultant of these two growing forces may be resolved in a diagonal direction cephalolaterad by the parallelogram of forces at the region of the shoulder-joint. The humerus, adducted ventrad to the thorax, rotates inward at the shoulder-joint, due to the relationship it bears as a point of insertion for the premuscular tissue of the pectoralis major muscle. This premuscular tissue is subdominant in formative growth, and as a consequence it is drawn out in traction along the progressively formed resultants caudocephalad, resolved in the parallelogram of forces produced by the clavicular and sternal forces of dominant growth.

The rotation of the fore-limb is due to two factors: first, the accelerated longitudinal growth of the vertebrae and the transverse growth of the ribs are also involved; second, the subdominant growth of the embryonic musculature. The stretching of the mesenchyme because of differential growth leads to the reactive pull causing limb rotation. The dynamics involved in the tendon formation of the pectoralis major muscle is of main concern in this report.

The first active line of tug will be from its most distal point of origin to its most proximal point of insertion on the humerus. As soon as the humerus is adducted corresponding to this initial

muscular tug, successive lines of traction and resulting contraction are established caudocephalad at the origin (figs. 1 to 6, *a, b, c, d, e*) of the muscle and cephalocaudad at its insertion (figs. 1 to 6, *a', b', c', d', e'*). These progressive lines of muscular pull are produced by the accelerated growth of the related skeletal segments (clavicle, sternum, ribs, and vertebrae). As long as the humerus is free to rotate, the inevitable fore-limb rotation, in embryos 15 to 40 mm. in length, is the resultant. With the humerus completely adducted across the ventral aspect of the thorax, the embryonic muscular fasciculi are lineated along direct lines of adequate traction from origin to insertion. In this condition there is no twist in the tendon of the pectoralis major muscle. Those fibers arising from the clavicle and cephalic aspect of the sternum have a more distal insertion on the humerus, whereas those arising from the caudal aspect of the sternum and lower ribs have a more proximal insertion in the humerus (fig. 5). This relationship is the consequence of the successive induced lines of tractional stress in the premuscle mass by the rapidly growing skeletal segments.

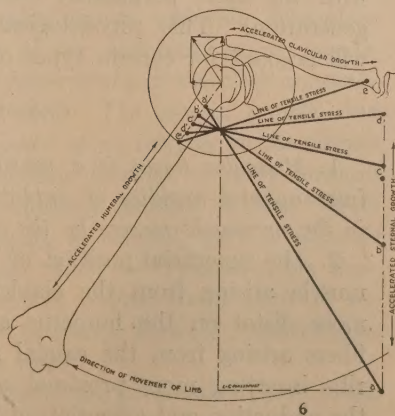
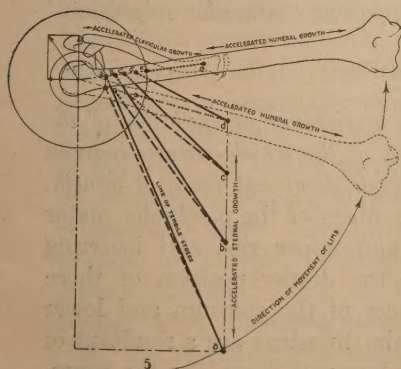
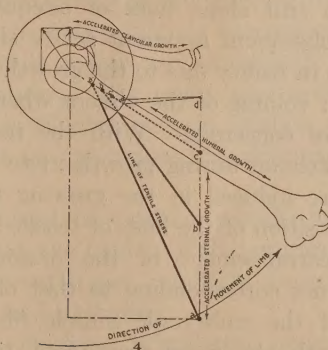
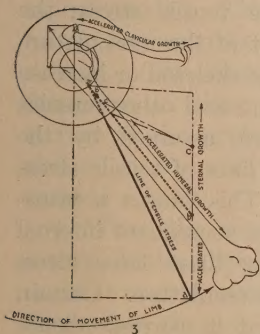
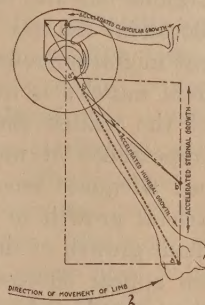
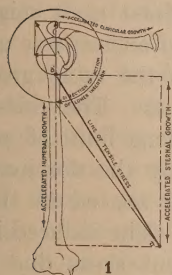
The limb is not fixed in this position by any means. There is active movement of the appendage due to muscular activity. The initial single sheet of the tendon acquires its overlapping characteristic with the interaction of traction and contraction manifested by the pectoralis major muscle. The synergists of the pectoralis major muscle abduct and extend the humerus in contrast to its former position of adduction and flexion. With this active interplay of opposing muscles the humerus is alternately in one position and then another. The abducting and extending activities of the synergists of the pectoralis major muscle cause the overlapping of its tendon (fig. 6). When the humerus is extended and drawn parallel to the side of the body or abducted the fibers arising from the clavicle and cephalic aspects of the sternum and upper ribs will inevitably assume a superficial relationship to those that arise from the caudal aspect of the sternum and lower ribs. The direction of the muscular fasciculi is determined by the lines of tensile stress induced in the premuscle mass by the accelerated growth of the related

skeletal segments. There is a tendency to untwist the overlapped condition of the pectoralis major tendon when the humerus is parallel to the clavicle in a flexed and ventro-adducted condition. But with the opposing activity, extension and abduction produced many times by the synergists, the architecture becomes fixed.

The successive appearance of new skeletal muscular components is directly determined by the progressive extension of the accelerated growing skeletal segments related to the muscle involved. One unfortunate term has been used in the literature of myogenesis, namely, '*muscular migration*.' Migration, in this sense, means an active movement on the part of the muscles from one region to another. The appearance of muscle fibers in

Figs. 1 to 6 This diagram first illustrates the humerus parallel to the lateral line of the thorax (fig. 1). The important skeletal zones of dominant accelerated growth are the sternum and clavicle. The growth of the ribs and vertebrae are also involved. The zone of subdominant growth is the pectoral premuscle mass. The sternal growth exerts its tension on the embryonic pectoralis major muscle in a longitudinal direction, that of clavicular growth in a lateral direction. The resultant of these two skeletal forces of growth may be resolved in a diagonal direction cephalolaterad by the parallelogram of forces at the region of the shoulder-joint. The fibers of the pectoralis major muscle during the period of fore-limb rotation progressively manifest their active tug from the distal to the proximal points of origin. This origination corresponds to the proximal-distal points of the insertion, as the humerus is adducted ventral to the thorax successive muscular fibers are formed.

Figs. 2 to 6 *a*, *b*, *c*, *d*, and *e* (origin); *a'*, *b'*, *c'*, *d'*, and *e'* (insertion). The fibers are stimulated by tension and assume their characteristic position because of the accelerated growth of the sternum, clavicle, and also ribs and vertebrae. When the humerus is parallel to the clavicle in a ventro-adducted position after the initial completion of fore-limb rotation, no overlapping of the tendon of the pectoralis major muscle is seen (fig. 5). When the synergists of the pectoralis major muscle extend the humerus and abduct it to a position parallel to the lateral line of the thorax, there is an inevitable overlapping of those fibers originating from the caudal aspect of the thorax and lower ribs by those which originate from the clavicle and upper ribs in the tendon of insertion (fig. 6). The latter group of fibers are consequently superficial in the tendon and have a more distal insertion on the humerus; the former are more deeply placed in the tendon and have a more proximal insertion. It is clearly apparent that accelerated skeletal growth not only induces the intermittent tension causing the genesis of the pectoralis major muscle, but the architecture is explicable only in terms of this accelerated skeletal growth plus the rotation and ventro-adduction of the humerus. The subsequent extension and abduction of the humerus cause the overlapping of the tendon of insertion of the pectoralis major muscle.



successive regions like the initial growth of the pectoralis major in a cephalocaudal manner cannot be defined as a migration. The apparent migration considered as due to an intrinsic activity of the skeletal muscles is in reality due to the rapid growth or extension of the related skeletal components. The consequent successive appearance of muscular fibers along lines of progressively formed adequate tension is caused by the dominant, accelerated skeletal growth of the components related to the area of subdominant growth of the mesenchyme. The induced lines of tensile stress appear successively in different areas due to the dominant accelerated, skeletal growth. All musculature is formed in situ along lines of adequate intermittent tensile stress; the subsequent apparent active shift on the part of the musculature is in reality due to the growth in length of the skeleton or increase in volume of the viscera where the diaphragm and other muscles are concerned. With the more definite form assumed by the skeleton during growth more definite direct lines of tensile stress are induced in the growing musculature. This causes a transposition of the lines of tensile stress; there is a consequent internal rearrangement of the muscular fibers along these later stress lines corresponding to that of the mature musculature. Certain of the embryonic muscle fibers not lineated in correspondence with the more permanent tensile stress lines will undergo degeneration. This physiological degeneration of muscle fibers will account for certain types of apparent 'Muscular migration.'

CONCLUSIONS

1. *Muscular tissue is a sensitive tensiometer or indicator of the intensity and rapidity of application of the tensile stresses induced in the premuscle masses by the extrinsic dominant zones of growth.*
2. The superficial position of the fibers of the pectoralis major muscle arising from the clavicle and upper ribs and inserting more distal on the humerus, and the deeper position of those fibers arising from the caudal aspect of the sternum and lower ribs inserting more proximal on the humerus are a resultant of the abduction and extension of the humerus caused by the syner-

gists of the pectoralis major muscle. This results in an overlapping of the tendinous insertion of the pectoralis major muscle.

3. In the inception of the fore-limb rotation there is no overlapping of the tendinous components of the pectoralis major, when the arm was successively flexed and ventro-abducted. Fore-limb rotation is caused by the accelerated growth of the clavicle, sternum, vertebrae, and ribs. This successive, accelerated growth draws out in traction the related muscles along the resultants of the parallelogram of forces. The muscular fibers are lineated along the adequate tensile stress lines induced in the premuscle masses. The clavicle grows laterad; the sternum cephalocaudad. The resultant humeral force is cephalolaterad, from below, but caudolaterad from above as the limb is successively rotated. The fibers of the pectoralis major are stimulated to formation in these successive positions. The origin and architecture of the pectoralis major is intelligible only in relation to skeletal growth and the intermittent tension induced by dominant skeletal growth.

4. When the arm originally assumed its position parallel and ventral to the clavicle in limb rotation the tendon of the pectoralis major was not overlapped. This overlapped condition became fixed when the arm was extended and abducted.

5. *The dynamics of myogenesis involving intermittent traction and contraction (work) of differential growth not only determines the genesis but the architectonics of the pectoralis major muscle. The genesis, growth, maturity, and hypertrophy of muscle is a quantitative property of its function or work. The genesis, growth, and hypertrophy of muscle is a direct index of an increase in the tension within definite limits. When the tension becomes relatively constant at maturity, the muscle remains relatively constant in size. With an increase in tension, after maturity is reached, there is increase in muscular work. Since Muscular Work = Tension times Contraction, an increase in tension within certain limits leads to increased function. This in turn produces muscular hypertrophy because of the increased metabolic rate.*

6. The overlapping of the tendon of the pectoralis major muscle is produced by the lateral replacement of the humerus in turn

caused by the synergists of the pectoralis muscle. The greater part of the pectoralis major muscle is formed caudocephalad during the period of the fore-limb rotation in human embryos 20 to 35 mm. in length. This myogenesis is the product of an intermittent tensional stimulus exerted in a cephalolateral direction as the resultant force of the lateral clavicle and cephalic sternal forces of growth.

I wish to thank Eugene Haug, consulting engineer, Milwaukee, and Leo Massopust, department artist, for their help in this work.

Abstracted by Edward F. Malone, author. University of Cincinnati.

Sharpening microtome knives.

This article discusses the nature of the edge of knives and the action of abrasive substances under different conditions. The preparation is described of hones sufficiently large to permit the knife to be sharpened without any portion projecting beyond the edge of the hone. The surface of the hone is leather glued to stone; the leather is then finished plane and treated with castor oil and grit. The methods of refining and testing grit are described at length, together with the proper care and use of the hones. Types of suitable honing backs are described and illustrated. The double concave knife is recommended, together with a large knife-holder adjustable for tilt. The method described is suited to the production of an edge uniformly good throughout its entire length rather than one of the very highest perfection.

SHARPENING MICROTOME KNIVES

EDWARD F. MALONE

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FIVE FIGURES

In this article I propose to discuss the imperfections in design of microtome knives, the methods of making faulty knives serviceable, and especially the principles and methods of sharpening such knives as permit of being sharpened on a hone. That the edge of knives is usually unsatisfactory is a fact that permits of no discussion; the expression of more general complaint is due not to the absence of dissatisfaction, but to the belief that a good edge can be obtained only by an expert. While this belief is in general correct, the services of an expert are necessary only because he sharpens imperfect knives by methods requiring great skill. I propose to describe a method by which an excellent edge may be obtained without the use of great skill and which demands only the exercise of care and intelligence. The edge so obtained is not of the highest refinement, but is characterized by uniform excellence throughout its entire length.

The edge of a knife is formed by the intersection of two narrow plane surfaces, the cutting-facets; the base of the included wedge is continuous with the body, and each facet with a surface of the knife. The facets result through the formation by the hone of fine oblique parallel scratches, and these scratches result in a serration of the edge with the teeth directed towards the heel of the knife. To facilitate rapid cutting, the knife rests upon the hone only on its narrow cutting-facet and a second broader facet situated on the surface of the knife immediately adjoining the back; both of these facets or supporting surfaces lie in the same plane. To insure the knife's resting upon these two surfaces of limited area, each side is ground concave or else the back is elevated by the attachment of a special honing back.

In the process of sharpening it is thus evident that the edge is not formed directly, but is the product of the formation and development of the two cutting-facets, and that any defect which the edge possesses is the expression of some defect in one or both of the two intersecting planes by which the edge is formed. Since the cutting-facets are not smooth surfaces, but are marked by

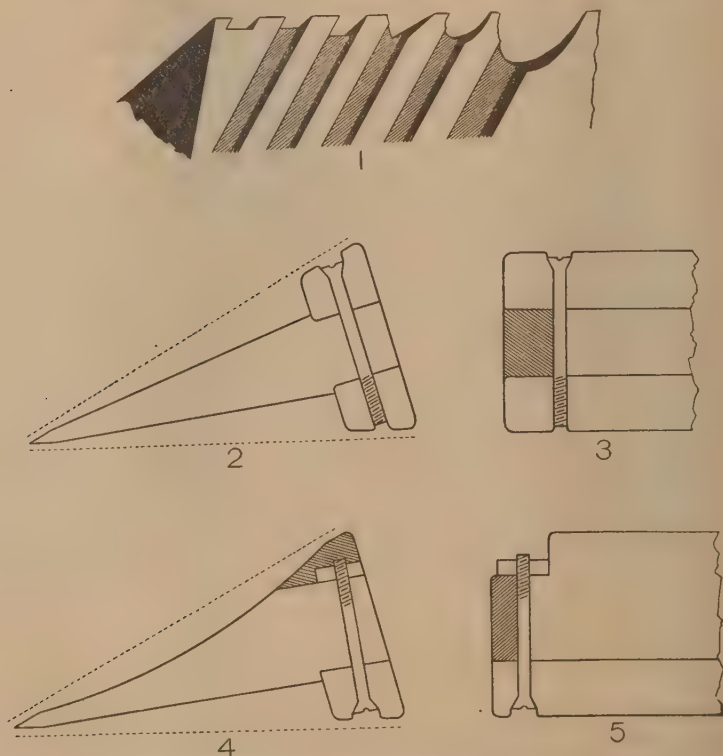


Fig. 1 Theoretical diagram drawn from wood model. From left to right a scratch intersecting with (1) near facet, (2) far facet, and (3) with another scratch; also scratches resulting in (4) a small V-shaped break, (5) a small U-shaped break, and (6) a true gap.

Figs. 2 and 3 Wedge-shaped knife and back. Shaded region in figure 3 represents slot; note rounded corners.

Figs. 4 and 5 Planoconcave knife and back. Shaded region in figure 5 represents slot.

oblique, parallel scratches, it follows that they cannot intersect in a smooth linear edge. The scratches upon both facets do not necessarily occur opposite one another and often cut the surface of the opposite facet after crossing the median plane. Since the scratches are parallel to the surface of the facets, their intersection with one another or with the opposite facet is at the same angle as that of the elevated portions of the facets. It is then evident that since it is impossible to form two perfectly plane surfaces intersecting to form a linear edge one should use every effort to approach this condition as completely as possible, to reduce the size of the scratches to a minimum while retaining the generally plane facets, without which a rounded edge results. An important reason for uniformly fine scratches is that when a deep one occurs the local pressure on the facet is tremendously increased, resulting not merely in a deeper serration, but also in an irregular breaking away of the steel near the edge which further deepens the gap (fig. 1).

The question arises as to why it is impossible to form two plane facets, polished free from even the finest scratches and intersecting in an edge free from serrations. Theoretically, this is possible by suitable stropping, but practically the lessening of the serrations is due largely to direct rounding off of the edge rather than to the primary removal of the scratches to which the serrations are due. In addition to the tendency to round off the edge, the strop is a source of further serious injury to the knife, the nature of which will be considered later.

Intersecting plane cutting-facets may be formed by means of a fine wheel or a fine plane hone. The wheel cuts the knife on the cutting-facet alone, while the plane hone cuts in addition a second facet adjoining the back of the knife. Since this second facet lies in a continuation of the plane of the cutting-facet, these two surfaces will stabilize the knife, and however often it be sharpened on a plane hone the angle and line of intersection of the cutting-facets will necessarily remain constant. Of course a knife could be thus ground on a wheel by passing the knife in a fixed plane tangent to the periphery of the rotating wheel, and the sharpening could be continued without interruption on a plane hone, since

a knife so ground would have on each side two plane facets lying in the same plane. It is hard to see why knives should not be so ground, but as a matter of fact they seldom are; usually the cutting-facets are ground without relation to the back, and this is unsatisfactory because the new edge is not sufficiently keen and cannot be improved on a plane hone and when dull must be re-ground on a wheel. This independence of cutting-facet and back is such that when the edge is placed in contact with a plane surface only one corner of the back will rest on that surface, the other corner failing to touch in many cases by as much as several millimeters; on the opposite side of the knife the opposite corner of the back is apt to fail to touch. Such a knife must be reshaped by grinding, and after the cutting-facets have been developed on a plane hone together with the secondary supporting facets, the cutting-facets should be narrowed by concave grinding. Of course a knife should be in this condition when purchased, but since they are usually not to be obtained in this condition they should be sent to be properly finished.

Accordingly, although the scratches made by a fine wheel are too coarse and cannot be reduced on a plane hone, the wheel is of great use in generally reshaping a knife, in removing large gaps, and in narrowing cutting-facets through concave grinding. The value of the wheel for this work lies in the uniformity and rapidity with which it cuts. On the other hand, the cutting-facets must be formed on a plane hone and the rate of cutting must be exceedingly slow, since a very coarse hone or the use of any external pressure will cause deep scratches and gaps. Since the steel must be removed very slowly by means of minute and uniform scratches on the cutting-facets, the process can be shortened only by keeping these facets narrow, thus reducing the amount of steel to be removed. By keeping the facets narrow a minute amount of steel can be delicately yet rapidly removed, and the keenness of the edge restored by a few strokes; theoretically to employ wide facets means lengthening the work from minutes to hours or even days, but practically it means failure, for in such a prolonged process accidents are almost sure to occur, thus indefinitely postponing completion. But there is a minimum

width of cutting-facet below which insufficient support is furnished, resulting in a rounded or broken edge. This minimum width depends upon the total pressure exerted upon that portion of the cutting-facet in contact at any instant with the hone, and since all external pressure must be avoided one needs only to consider pressure due to the weight of the knife. The most important factor is the angle formed by the cutting-facets, for as the back is elevated a greater proportion of the weight falls upon the cutting-facets. A thick, broad knife weighs more per unit of length, but this is counteracted by the shifting backwards of the center of gravity. A long knife on a narrow hone will be supported by less than the total surface of the cutting-facet, and even if the hone is so large as theoretically to support the entire surface of the facet, yet due to inequalities in the surface of the hone a long knife will exert greater local pressure than a short one, and coarse scratches with resulting gaps are always due to excessive local pressure. Practically I recommend a cutting-facet about $\frac{1}{2}$ mm. in width, with a minimum width of $\frac{1}{4}$ mm. and a maximum of 1 mm.; when the width exceeds the latter measurement, the cutting-facet should be narrowed to about one-half millimeter by removing its rear portion through concave grinding.

Narrow cutting-facets are obtained either by a double-concave grinding or, in the case of a wedge-shaped knife, by elevating the back through attaching a special honing back. The latter type of knife permits the facets to widen more rapidly than in the case of the double-concave knife and presents other difficulties to be considered later. The wedge-shaped knife is by some supposed to be more rigid than the double-concave type, but one should not overlook the fact that the wedge-shaped knife with attached honing back is usually sharpened at a more obtuse angle; to obtain the same angle of edge in knives of the same width the back of the double-concave knife should have the same thickness as that of the other type with its honing back attached. The rigidity of a knife depends almost exclusively upon the angle of the edge, which should be a little more obtuse than is required by the work which the knife has to do, and with equal angle of edge there can

be no essential difference in rigidity between a thin wedge-shaped knife and a thick wedge-shaped knife subsequently ground concave. Moreover, in comparing types of knives one is apt to overlook not only the angle of the edge, but those manipulations of the knife by which its tilt and slant are adjusted to the thickness of the section and the nature of the tissue. The only knife-holder worthy of consideration is one adjustable for tilt and large enough to hold a thick knife; the unfortunate replacement of the complete knife by the thin knife with removable back is encouraged by the small knife-holders usually supplied. While it may be possible that for certain kinds of work a plane surface is superior to one which is concave, yet this possible advantage can never compensate for an inferior edge, and a good edge is more easily obtained on a double-concave knife. Bearing in mind the fact that a double-concave knife should be ground from a wedge of steel whose angle, and therefore the angle of the finished edge, is suitable for the work required of it, I unhesitatingly recommend this type of knife. And I believe that the objections to it depend in large measure, if not entirely, upon the use of an edge of unsuitable angle and upon improper adjustment of the knife.

When the edge of a knife is too acute, the only way to increase its angle is to employ a special honing back. The honing backs in the market are useless, since they fail to stabilize the knife when the latter rests upon a cutting-facet, often shift position during honing, and cannot be replaced on the knife in the same position. I have had much experience with them and am convinced that those on the market are failures. This inferiority of design and manufacture of honing backs is not the fault of the manufacturer, but is due to the unexacting demands of their customers who have never considered honing in the light of a rational procedure, but one so closely allied to the supernatural as not to exclude the hope of obtaining a good edge by the formation of two rounded shifting facets determined by the unstable back. Moreover, these backs are supposed to fit any knife of a certain size and type, regardless of the individual peculiarities of knives due to warping, and often a back is not reserved for one particular knife, but is used on several. Until a more discriminating demand arises,

one cannot expect manufacturers to regrind their knives true after tempering and to supply each perfectly symmetrical knife with a honing back which will fit perfectly and can always be attached in exactly the same position. In fact, I recommend special honing backs only for expensive knives which one happens to possess and which cannot otherwise be suitably sharpened. No honing back can make a knife equal to a double-concave knife, and accordingly the latter is the only type which should be purchased. A honing back should not be attached to a double-concave knife; if the edge of such a knife is too acute, save the knife for suitable tissue; for using an oblique stroke, the increased acuteness of the effective angle of the edge will lessen the danger of injury, and under these conditions such a knife may be ideal. The two types of knives which must have honing backs are the wedge-shaped and plano-concave; the former should have an elevating strip on each side and the latter on the plane side only.

In connection with honing backs the following principles should be observed (figs. 2 to 5). Those portions of the knife and elevating strips which lie in contact should be ground perfectly plane and each strip should rest against a slight shoulder on the knife; this insures a perfect fit necessary for stability and accuracy in reassembling. The two surfaces of the knife in contact with the strips are best ground parallel to one another to insure accuracy in drilling the strips as they can be drilled while clamped together. The knife cannot be drilled and therefore the screws are made to lie in narrow slots, at each end of the knife, ground perpendicular to its two parallel surfaces; if a third screw seems advisable, a slot could be ground for it in the middle of the back of the knife. The elevating strips must be drilled and tapped and therefore of soft steel or iron, the tendency to wear away rapidly being balanced by the relatively large surface. These strips should be of sufficient thickness to give, after they are reground, the desired angle. Due to the grinding away of the surfaces of the knife, these strips are thick enough to avoid vibration and bending and to allow countersinking of the machine screws. The machine screws should have a diameter of $\frac{1}{8}$ inch and be flat-headed, since this type can be more accurately countersunk. The slots

should be so deep that the head of the screw will be removed from the end of the knife by at least 1 mm. When attached the elevating strips should be so ground that their surfaces lie in the same plane as the edge, and after the formation on a hone of the cutting-facets the latter should be narrowed by lightly grinding away their posterior border. All edges and corners are rounded off to protect the hone. Of course each knife must have its own back and each should be identified by number, and the pieces marked to insure proper assembling. In case of the planoconcave knife, the back of the concave side is ground parallel to the plane surface, but only at each end. While I have as yet never had the equipment to make such a back, I have prepared much cruder ones which are satisfactory, and accordingly do not hesitate to recommend the back described above. As previously stated, such a back will often make an expensive knife usable, but in ordering new knives double-concave knives of proper angle should be specified.

Assuming that we have a double-concave knife or a knife with a honing back which remains rigidly in place and can always be replaced in exactly the same position, we may now consider hones and methods of sharpening. From the nature of the cutting-facets it follows that a hone should be plane and should remain so, and that if it is large enough to permit one to keep the entire knife always upon the hone the process of keeping the facets plane will be made more accurate and much easier to accomplish. For if the hone is plane and cuts finely and if the entire length of the facet rests upon the hone little skill is required to produce a good edge. Assuming the size of the desired hone to be 1 foot by 2, it is evidently impossible to secure a hone of the usual type in this size, and artificial abrasives which can be made into a stone are too coarse.

At first I tried sharpening on plate glass with fine carborundum powder and water, and after several years found why this method can never be successful. The facets appear very evenly and finely frosted, but partial polishing reveals the presence of deep scratches and numerous pits extending deep into the steel. Unless of extreme fineness a freely moveable grit between two moving

hard surfaces will always cause the formation of pits and gashes under cover of the delicate surface finish. This method has been successfully used by Funck (*Zeit. f. wiss. Mik.*, 1910), who employs an extremely fine grit, thus limiting the method to the formation of the final edge; however, the danger from the accidental presence of even relatively fine foreign grit is always present. Reflecting upon the difference in behavior between a movable grit and the fixed grit of a stone, it occurred to me that the complete fixation of the grit was far from ideal; for instance, the tendency of the stone to pull out particles of steel and the danger of injuring the edge on the hard stone in case of a slight accident. As a result I decided to try the effect of partial fixation of the grit, embedding it in a surface which would not hold it firmly against excessive resistance, and covering the surface with a thin layer of grit which would pack into a self-leveling abrasive surface unyielding under the weight of the knife. The material selected was leather treated with castor oil and has justified all expectations.

The leather surface must have a rigid support which will not warp, and accordingly wood cannot be used. I use any soft stone which can be easily cut with a saw, ground plane, and the corners and edges finished with a rasp, while sufficiently porous to hold glue. The upper surface of the stone is ground plane after well rounding off the corners and edges to prevent breaking. Three such stones should be prepared, since a coarse, a medium, and a fine hone are necessary. The two finer hones should measure 8 inches by 24, while the coarse hone should measure 12 by 24 inches. Before handling the leather all grit must be removed from the stone, hands, clothing, and surroundings, since any grit once embedded in leather can never be removed. For leather I use a good grade of cowhide of uniform thickness and texture. In clean surroundings the leather is cut to the proper size, leaving a margin of stone at least 1 cm. wide and the corners of the leather cut off round to correspond to those of the stone, since sharp corners tend to become detached. A plane surface is selected of suitable size and such a nature as to permit the use of large clamps, and is covered with a piece of clean paper to prevent

the leather from sticking to the surface beneath the paper. The leather is placed dressed side down upon another piece of paper in a different location and both the leather and the surface of stone given a coat of hot glue of a good grade; from now on one must work quickly. The two coated surfaces are placed in contact and the leather smoothed out, pressing out firmly the excess of glue which is wiped off with a damp cloth, care being taken to remove any glue on the upper surface of the leather. The hone is then placed carefully, leather surface down, on the paper-covered surface prepared for it and the clamps carefully and uniformly tightened to counteract the tendency of the leather to shift its position.

After twenty-four hours the clamps are removed and the leather surface carefully dressed with a plane. The plane should be at least 15 inches in length, very sharp and free from gaps. The plane must not cut deeply, since this might cause the leather to tear or crack, and should be moved in all directions, especially along the diagonals, frequently turning the hone end for end, so as to insure a plane surface. When the surface is plane the edges and corners are slightly beveled and the surface finished by lightly shaving with a sharp microtome knife. This knife must lie flat on a plane surface and is lightly moved edge first in different directions in such a manner as to render the surface plane and evenly finished. The leather surface should have a fine velvet finish and is now ready for the oil and grit. When not in use each hone should be covered with its own properly labeled dust-proof top of wood or cardboard.

The leather surface of each hone is treated with castor oil, which should be uniformly distributed and thoroughly rubbed in. More oil is added and upon the addition of the appropriate grit the resulting paste is rubbed into the leather; fresh grit is added from time to time. The rubbing should be vigorous and rags or paper should be used. As the paste becomes thick the velvet surface of the leather begins to come away with the paste and the leather becomes smoother and firmer. This thick paste fills the pores of the leather, and when the hone is in use will form a thin firm layer over the leather which should be covered in turn by a

very thin layer of dry grit. Do not add too much oil, since the excess must be removed and this is tedious and requires the use of much grit. After a day or two when the oil has soaked in the excess of oil is removed from the leather by repeated and vigorous rubbing with many clean pieces of paper toweling. This process should be thoroughly carried out so as to avoid the necessity of too frequent repetition. Before use the leather surface is brushed off, rubbed with the palm of the hand to free it from foreign particles, and a little grit added, which is rubbed in by hand and smoothed with the palm. Beginning from the time that the grit is first rubbed into the hone, each hone should rest on a separate table in surroundings free from coarse grit and should have its own dust-proof top. Since the surface is always smoothed with the palm, the hands and finger-nails must be carefully cleaned, especially when one is to use the finer hones, and one should always work with bare forearms. Honing backs must be removed from knives and each carefully washed with soap and water and then dried, paying especial attention to recesses in which grit may collect; this is one of the disadvantages of such backs. An ideal source of contamination is the greasy castor oil bottle and its cork, which should be protected from all grit and carefully cleaned before using. The necessity of care in protecting the fine hones is imperative, since coarser grit once imbedded can never be removed; it can, however, by repeated cleaning be rubbed into the leather and kept covered with fine grit.

The nature of the surface of the leather hones must be carefully considered; the surfaces of the three hones differ only in that the finer the grit the more delicately it cuts and the more readily it packs into a compact layer. The addition of castor oil to the leather changes the hard leather with superficial velvet finish, a finish which would round off the edge, into a dense, sticky, homogeneous mass. Thus the velvet surface is either rubbed off or else incorporated with the rest of the leather, and the softened pliable leather is more receptive to the grit. Without the presence of grit oiled leather will cause a knife to adhere tightly and is absolutely useless. This tendency to cause the knife to adhere continues to reappear to a slight extent after the grit is added,

and when slight may be overcome by rubbing up the grit and redistributing it with the palm of the hand. But in new hones frequent rubbing off of the excess of oil with paper is necessary. The carborundum grit fills up the pores of the leather and renders it firmer, and while rouge on oiled leather will cause the knife to adhere hopelessly, carborundum grit if properly manipulated will form a surface over which the knife glides without any pressure other than that due to its own weight. The dry grit should form, above the firm paste, a definite layer, but one whose thickness is almost inappreciable. When gently roughened up with the palm, the grit, as the knife passes over it back foremost, fills in any irregularities in the leather and automatically forms a plane surface. The leather is inelastic, but is readily dented through pressure exerted by a sharp object, such as a corner of the knife or a finger-nail. These marks may be removed by rubbing or forcibly ironed out by any object having a straight rounded edge, such as the edge formed by the back and main surface of the knife, but such depressions are harmless unless excessive and soon become filled with dense paste or dry grit.

This soft, homogeneous consistency of the leather is the source of advantages not found in case of a stone surface. Under the weight of the knife the leather is absolutely unyielding, the surface being as dense as the rest of the leather, and in this respect it is equal to a stone surface, while in other respects it is superior. The leather does not cause the knife to vibrate, it always remains plane, and in case of slight accidents the soft leather will not hold the grit firmly, thus avoiding great injury to the knife. Such accidents as slightly elevating the back of the knife, the presence of foreign particles on the hone, or striking the edge of the knife against the edge of the hone, while very serious in case of a stone surface, cause relatively little damage in case of the leather hone. In other words, oiled leather is as harmless a substance as will support a knife without yielding under its weight, while the particles of embedded grit are capable merely of individual action which is limited, in the event of unduly increased local or general pressure, by the ability of the particles of grit to give way; whereas a stone acts both through its rigidly fixed

individual particles and also through the unyielding mass which these particles form. Moreover, unlike stone or plate-glass, leather cannot give off any hard particles such as are a source of constant danger in using a hard surface. The ease and certainty with which a good edge is obtained on a leather hone is the result of avoiding or reducing to a minimum the effects of those frequent slight accidents which are often undetected.

The nature of the grit and the method of refining it will now be considered. The entire support of the cutting-facet of the knife is equal to the combined support of all the individual particles of grit with which the facet is in contact. In the case of coarse grit, the tops of these particles are relatively far apart, thus offering relatively less total support to the facet and thereby causing the facet to exert a relatively greater local pressure on each particle; moreover, the greater height of these particles permits them to cut more deeply into the facet before the latter receives additional support from deeper-lying particles. In the case of a fine grit, the total area of contact with the facet is greater while this increased surface of contact is formed by a much larger number of particles, and consequently the local pressure on each particle is decreased, thus causing them to cut less deeply. Grit cuts most rapidly when used upon a hard surface, such as a plate of glass, less rapidly when held together by a binding substance, as in the case of a stone (except when particles of grit become dislodged), and least rapidly when used upon a surface which will allow the particles to sink in, thus tending to equalize local differences in pressure. A large particle of grit will cause much less damage when used with other particles of the same size than when used with finer particles, since in the latter case the entire support of the facet is furnished by the single large particle which extends above the general surface of the fine grit; moreover, the fine grit will form a delicate edge so that under these conditions a large particle will not only cause a deeper scratch, but will break away the edge to a greater extent. Isolated coarse scratches with their accompanying gaps are merely the result of abnormal local pressure, of local rapid cutting during a stage of honing wherein the general rate is slow; whereas during a stage of honing character-

ized by general rapid cutting the roughness of the edge must be considered normal. Accordingly, during any stage of honing the size of the scratches should be nearly constant, larger or smaller according to the rate at which the steel is removed. In other words, however coarse or fine the grit, it will cut with maximum rapidity and with minimum injury when its particles are of uniform size.

Carborundum grit should be used, since in addition to its extreme hardness it spreads into a smooth layer without the tendency to form isolated lumps; a 5-pound can of 'FF' and also of the 'Sixty minute' powder should be procured. It is absolutely essential to refine these commercial powders, and when refined the coarse or FF powder is used for the coarse hone, while the Sixty minute yields, after grinding it into a finer powder, two grades of powders for the medium and fine hones, respectively. The refining is carried out by settling in water and the result controlled under the microscope with the aid of a micrometer eyepiece. Under each of two conditions some of the grit will fail to settle in water, for dry grit will form a surface film over the water and air bubbles will carry a film of grit upwards; these circumstances, while of no importance during the elimination of particles too small to be of use, involved most serious consequences when the particles to be eliminated are too coarse for use. Accordingly, a little of the dry powder should be placed in a mortar, and when thoroughly mixed with water it is poured into a liter measuring cup or pitcher, which when filled is caused to overflow by the addition of water and the floating grit and air bubbles brushed off. During subsequent operations, whenever coarse grit is to be eliminated, the bottles should be filled to overflowing immediately before allowing contents to settle, and the surface grit floated off before introducing the siphon; moreover, as the surface falls in the bottle it may regain a film of grit from that adherent to the sides so that the upper centimeter of the contents should never be siphoned off. One must remember that the position of a particle of grit which has been allowed to settle for a definite time depends not only upon its weight, but also upon the point from which it started; therefore the separation can be

accomplished only by repeated settling and siphoning with the use of many changes of water. All particles above a certain size will settle each time, but will be mixed with many useful smaller particles, and after all the latter have been recovered by repeated settling all the useful particles can be made to settle each time, but must be washed free by repeated settling from those particles which are too small to use. Another source of contamination is due to the disturbing of the settled grit through the current set up by the siphon, so the end of the tube should never closely approach the layer of settled grit and the stronger the stream the greater should be the distance; this is naturally of greater importance in the case of the finer grades. Finally the grit should be dried at room temperature, since if dried over a flame it will unite into dense masses which will not disintegrate upon rubbing.

The FF carborundum powder contains relatively few particles too coarse to use upon the coarse hone, but many which are too fine, including a considerable amount of what appears to be rouge or else mud; on account of this contamination the finer particles should not be saved for the finer hones. In preparing the grit for the coarse hone I reject all particles large enough to settle 10 or 12 inches in one-half minute, and reject all those too small to settle 5 or 6 inches in one minute. It is best to begin by removing the excessively small particles by thoroughly rubbing up the grit in a liter cup of water, allowing it to settle one minute, and pouring off half the contents; this is repeated until the water comes off clear. After this preliminary cleansing, the grit is poured into a bottle 12 or 15 inches high holding at least a gallon, and by repeated settling all grit siphoned off except that too coarse to use. Each time the contents is thoroughly mixed without undue formation of bubbles, the bottle filled to overflowing and the contents allowed to settle for one-half minute, after which the surface layer of grit and bubbles is again brushed off and the rubber tube introduced so that its inlet reaches within about 3 inches of the bottom. The contents is siphoned into a second gallon bottle, and as the pressure falls the inlet can be lowered, but never nearer to the settled grit than 1 inch. Since

the suspension is opaque, the tube should be so marked by adhesive tape that one can tell the position of its inlet; after each operation the tube should be washed inside and out. The above operation is repeated until all useful grit is removed from the first bottle, and since it is convenient to collect all of this useful grit in one bottle, each time this second bottle is filled its contents is mixed, allowed to settle two minutes, and siphoned off in the sink. After all the useful grit has been collected in the second bottle, the smaller particles must be eliminated by allowing to settle one minute and siphoning off into sink the upper half of contents, keeping inlet of siphon at a depth of 5 or 6 inches; after mixing the rest of contents and settling for the same time, the rest of the suspension is siphoned off. This is repeated until the water comes away clear. The grit should be well mixed and a sample examined under the microscope. The average size of particles should be 35 to 40 μ ; there should be few smaller than 15 μ , although some as small as 2 μ will appear. Particles 75 μ in length should not measure over 50 in width, but a few narrow ones may occur with a length of 150 μ . Since the particles are separated according to weight and since their shape varies, it is impossible to prevent a considerable variation in their long diameter. In this grade of grit the danger is in the presence of too many fine particles below 15 or 20 μ in diameter. After testing, the grit is filtered, washed with distilled water and preferably alcohol, and spread out to dry without the use of heat. It is kept in a properly labeled bottle with a cork stopper; a glass stopper must not be used.

The Sixty minute carborundum powder from which the medium and fine grades of grit are prepared is much cleaner than the FF powder and contains few particles too small to use, while the majority have to be ground still smaller. At first it is best to remove from the powder all particles small enough to use and then grind up the larger ones, since grinding is liable to eliminate too many of the size suitable for the medium grade of grit. The dry grit should be most thoroughly rubbed up with water until it goes into suspension, and great care must be taken to avoid surface films and films upon air bubbles; the inlet of the siphon

must be kept well away from the settled grit, since the latter is easily disturbed. The upper limit for the medium grade is a fall of 6 inches in two minutes and the lower limit an equal distance in ten minutes; but the latter time could probably with advantage be reduced from ten minutes to five or six, thus removing some of the finest particles. The upper limit of the fine grade is a fall of 6 inches in half an hour and the lower limit 12 inches in eighteen hours; but beyond doubt the grit would be improved by moving both time limits nearer together with an upper limit of one hour and a lower of six to eight hours. The settling process, filtration, and drying are carried out as previously described, remembering that whenever coarse grit is to be eliminated the bottle must be filled to overflowing before introducing the siphon and that the inlet of the siphon should never extend more than half-way to the bottom of the bottle. A large portion of the grit will be too coarse and must be ground before refining. A little grit is mixed with water to form a paste and ground between two glass plates which are then washed in a large pan of water, and the process repeated until the water is very milky; the added glass particles cut rapidly and are not objectionable. The milky water is poured into a large bottle and the coarse grit remaining in the pan saved for grinding. From this milky suspension the two grades of grit are prepared, first eliminating grit too coarse for the medium grade and then that which is too fine; from the latter the fine grade of grit is prepared. On testing the medium grade the largest particles should not measure more than $30\ \mu$ in diameter and there should be few below 8, while the average is about $17\ \mu$; as previously stated, I advise changing the lower limit from ten minutes to five or six. The fine grade should have no particle over $13\ \mu$; the smallest are about $0.5\ \mu$, and the average about 4. This variation is certainly too great, and I strongly advise changing the upper limit from half an hour to one hour, and the lower limit from eighteen hours to six or eight hours.

The general method of using the hones will next be described, followed by additional observations in the case of each hone. The fundamental fact to remember is that the knife should always

rest with merely its own weight upon the hone; all external pressure, however tempting, should be avoided, and the other source of pressure, namely adhesion, must be as carefully avoided through the proper care of the surface of the hone. The strokes employed are the same as in stropping, oblique strokes on alternate sides with the back moving in advance of the edge and the toe in advance of the heel; a handle must not be used nor should any portion of the knife project beyond the edge of the hone. Before each stroke the surface of the hone is lightly roughened up with the palm of the hand; this prevents the grit from packing into a hard layer and maintains a thin film of loose grit on the surface. Such a surface film of loose grit prevents adhesion of the knife to the hone and causes the latter to cut more rapidly. If sticky patches of grit appear, they can often be removed by vigorous rubbing of the entire surface of the hone with the hand so as to mix the oily grit with the dry, but at times the excess of oil must be removed by rubbing off the sticky paste with clean paper, after which fresh grit is applied and smoothed out with the palm. To prevent the formation of gaps through breaking the thin edge, the latter should, except in the case of the fine hone, be kept rounded and smooth. This is accomplished by lightly drawing the edge several times over the ball of the thumb covered with grit; the edge is removed before it can enter the epidermis. The edge is restored by about five double strokes on the hone, and accordingly the dulling should be repeated often, the frequency of the process depending upon the thoroughness with which it has been carried out.

The coarse hone is used when gaps exist, when the facets fail to intersect, or in any condition where much steel has to be removed, and when these defects have disappeared nothing is to be gained by longer use of this hone. The edge should be kept well rounded and smooth as just described, but if any doubt exists as to the intersection of the facets throughout their entire length, this dulling process should be discontinued until the edge along its entire length will cut a coarse hair; thereafter the dulling operation is resumed. Since the coarse hone should cut rapidly, the surface layer of loose grit should be thick, about 1 mm. in

thickness, and should be brushed off and replaced with fresh grit when it becomes oily and broken up into finer particles, thus slowing its cutting. Near the end of the operation the excess of grit should be removed, leaving a surface layer of loose grit about $\frac{1}{4}$ mm. in depth, and this grit should not be replaced by fresh; in this manner the facets will be more nearly plane and the knife in better condition to transfer to the medium hone.

The medium hone is used after the coarse and also to remove gaps which have appeared in the finished edge when these do not exceed a size barely visible to the unaided eye. As in the case of the coarse hone, it should have a surface layer of loose grit lightly roughened up by hand before each stroke. But since at this stage rapid cutting is of less importance than accuracy, this surface layer should be thin, about $\frac{1}{8}$ mm. in depth, and since the grit is relatively valuable it should be renewed only when necessary. Upon the gradual intermixture with oil, this grit will form a sticky paste more readily than the coarse grit, and accordingly more attention is necessary to maintain the thin surface film of loose grit so essential to prevent adhesion between the knife and hone. Greater care against contamination is necessary and before using the hone or when it otherwise appears advisable, the surface should be blown off and then lightly brushed off by hand, adding fresh grit when necessary. With the foregoing modifications the procedure is the same as usual, the knife moving obliquely under its own weight alone, back forward and toe in advance of heel, reversing sides and direction after each stroke, the surface grit lightly disturbed before each stroke, and the edge kept rounded and smooth. A medium polish appears on the facets, and the edge if not purposely dulled, as it should be at least at the beginning and the termination of the process, becomes very keen, but shows slight microscopic defects.

The fine hone is used after the medium hone and also before and after employing the knife for cutting. Its fine grit has the tendency to pack into a firm smooth layer which may cause the knife to adhere and its edge to break, and consequently a little fresh grit should be added after about twenty double strokes.

The fresh grit has the feel of talcum and should be frequently added in small quantities forming above the firm smooth layer of grit a film about as thick as a rather heavy film of talcum. If the hone is thus kept with a surface which has the feel of a film of talcum it will cut relatively fast, and the knife will glide over it with the greatest ease, showing no tendency to adhere. The method of sharpening is as usual, the surface layer being very lightly disturbed before each stroke, and all pressure on the knife as well as all careless manipulation being carefully avoided. This stage of honing should be carried out with deliberation, keeping clearly in mind that by the delicate removal of minute films of steel one is bringing to complete intersection two polished and perfectly plane facets; with such an ideal in mind any position of the knife which allows the facet to rest upon the hone in any manner other than one of light yet perfect contact will be recognized as the result of stupid carelessness in the manipulation of the knife. At first the edge should be kept dull as described, but later of course it must be allowed to reach its maximum keenness. After the imperfections due to the medium hone have been removed, the final finish is given to the edge by allowing the surface grit to pack somewhat, slightly disturbing it after every second double stroke, instead of after every single stroke. This gives an edge which is keener and more highly polished, but the tendency of the knife to adhere must not be allowed to go too far. It is evident that very careful refining of the grit is essential, for the perfection of the finished edge depends upon the nature of the grit.

The resulting edge is very keen, with highly polished facets, and should be used without attempting to improve it on the usual leather strop. Oiled leather without grit will cause the knife to adhere firmly, and if it is not oiled the surface is very apt to have rough places. Such rough places on soft leather will round off the edge, while on hard leather they will cause deep scratches and gaps to an extent almost unbelievable. A great deal of damage is done to knives by stropping, the nature of the injury depending upon whether the surface is sticky, fuzzy, or hard and rough. Even if a strop is hard and smooth it offers a surface upon which

a piece of lint or a particle of grit will do the maximum damage to the knife.

The edge should be tested by inspection under the microscope and by the manner in which it will cut a hair. Both of these tests are necessary, since in my opinion the keenness of the edge cannot be determined by inspection, which, however, gives most reliable information as to uniformity; while the manner of cutting a hair will give reliable information as to the relative keenness of large portions of the edge, but fails to indicate the extent or even the presence of sharply localized defects. The microscopic inspection is carried out by reflected light under the 16 mm. lens. A firm support, such as a book or a board, is placed on the stage and covered with a piece of clean paper upon which the knife is placed flat with the edge directly facing the light. The knife must not be moved over the paper, but only by moving its support over the stage; the light should always fall directly upon the cutting-facet from in front and above. In testing for keenness one should remember that while a rough edge will cut a coarse hair more readily than a fine hair, the reverse is true of a finely serrated edge, since it cuts a soft delicate hair with ease, but a coarse hard one only with the aid of pressure. An edge finished on the fine hone should throughout its entire length instantly sever a fine hair with extreme suddenness and smoothness. If the edge throughout its length meets this test of keenness and under low power of the microscope reveals no appreciable inequalities it is thoroughly serviceable. In fact, an edge which shows a few very minute gaps is for most purposes usable, since the loose grit tends to polish and round off and thus lessen such slight defects, an advantage especially apparent when the knife is used in the oblique position which permits such rounded and polished depressions to remain practically inactive.

A knowledge of the principles which underlie the process of sharpening is essential to success, and I have attempted to make their consideration sufficiently general to apply to all methods employing a plane hone. Since the principles and methods have been discussed somewhat at length, the impression may result

that the method described must of necessity be complicated. But the complexity lies in the operation of a large hone with partially fixed grit, a hone which through its lack of simplicity of operation automatically removes many sources of danger and thus permits the operator to attain success with certainty and with a minimum of skill. While the requisite skill has been greatly reduced, the delicacy of the edge is such that no method will assure good results in the absence of intelligence and care. Accordingly, it has appeared desirable to present a thorough discussion of the principles, aims, procedures, and errors, since their appreciation must always form the basis of intelligent effort.

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A note on the elastic membrane of the bronchial tree of mammals, with an interpretation of its functional significance

The network of longitudinal elastic fibers which forms a membrane in the tunica propria of the entire bronchial tree of mammals may be dissected off en masse from the fresh trachea and larger bronchi, and the specimen so obtained may be stained, cleared, and mounted. Stout in the trachea, and especially in the larger bronchi, this elastic membrane becomes thin and delicate in the terminal twigs, where it unites with the elastic tissue of the air chambers. Considered in toto, its form reproduces that of the entire bronchial tree. The limbs and trunk of the bronchial tree constitute a system of extension and contraction axes for the movements of expansion and contraction of the lung. The filling and emptying of any considerable area of lung is inseparable from the stretching and contraction of the airway. The elastic membrane is by far the most efficient part of the recoil mechanism of the bronchial tree, and is a very important, if not the most important, part of the recoil mechanism of the entire lung. Downward shifting of the lower part of the lung is necessary to free expansion of the upper part, and is associated with downward movement of the lung root and stretching of the bronchi and trachea. Pathological changes which tend to immobilize the lung roots or stiffen the bronchial tree will impair normal lung function, particularly in the upper lobe, and such changes may go far to account for the susceptibility of this part of the lung to tuberculosis.

A NOTE ON THE ELASTIC MEMBRANE OF THE BRONCHIAL TREE OF MAMMALS, WITH AN INTERPRETATION OF ITS FUNCTIONAL SIGNIFICANCE

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CURRENT KNOWLEDGE OF THE ELASTIC MEMBRANE

Attention has often been called to the fibers of elastic tissue which course in a longitudinal direction beneath the epithelium of the trachea and bronchi in all the higher animals, but so far as I am aware no one has sufficiently emphasized their prominence and importance in respiration. A typical text-book description is that of Jordan ('20, p. 302), who, writing on the trachea, reports as follows:

The tunica propria includes a thin inner layer of connective tissue which is richly supplied with small blood-vessels and infiltrated by many lymphocytes, and an outer layer of elastic tissue most of whose fibers are longitudinally disposed. The elastic layer begins in the region of the vocal cords in the larynx and is continuous below with the similar layer of the bronchial mucous membrane. Elastic fibers are more numerous in the trachea of the lower mammals than in that of man. A muscularis mucosa, a characteristic structure of the mucosa of the digestive tube, is lacking in the trachea. The elastic membrane occupies the position held by the muscularis mucosa in other organs.

Farther on (p. 309), speaking of the respiratory bronchioles, Jordan says:

The elastic fibers, derived from the elastic layer of the bronchioles, pass over to the alveolar walls in which they form a delicate network.

Other text-books (Landois, '92; Koelliker, '99; Luciana, '11, et al.) describe these fibers at greater or lesser length. The most comprehensive description of them, from the point of view of

comparative anatomy, is found in the well-known work of Oppel ('05), in which it appears that, though they are present in the respiratory tract of birds, and are even represented rather indefinitely in amphibians and reptiles, they reach their highest development in mammals. Within the mammalian group, too, they present some variation, but in all they show the same fundamental plan. So constant are they, and so conspicuous, that it is impossible to escape the conclusion that they must play a very important rôle in the physiology of the respiratory tract. The vascular subepithelial layer, rich in lymphocytes, is described by most authors and may be merged with the elastic membrane proper in some animals. The muscle of the bronchial tree is reported as situated just outside of the elastic membrane, the fibers being mainly circular in disposition, while the fibers of the elastic membrane are longitudinal. Beyond the muscle, again, are the cartilages of the airway, followed by a layer of adventitia. In the adventitia are numerous elastic fibers, but these do not form a definite membrane, and in direction they are irregular.

NOMENCLATURE

There appears to be no generally accepted term of designation for this system of fibers. Jordan, as we have noted, refers to this layer of elastic fibers collectively as the 'elastic layer' or 'elastic membrane.' Among the names which are used by the German writers are 'subepitheliale elastische Faserschicht,' 'elastische Längsfaserschicht,' and 'subepitheliale Schicht elastischer Fasern.' Since the fibers make up a system extending from the larynx to the outermost terminations of the bronchial tree, I shall refer to the entire system as the 'elastic membrane of the bronchial tree,' and shall consider it as extending from the lower end of the larynx, through the trachea, larger and smaller bronchi, and bronchioli to become continuous with the elastic fibers of the respiratory tissue of the lung. Often, for brevity, I shall refer to it merely as the 'elastic membrane.'

In the following pages I have limited myself to a consideration of these fibers as they occur in the higher mammals only, and particularly as they are found in the cat, dog, pig, and sheep.

APPEARANCE IN THE FRESH CONDITION

The elastic membrane of the trachea and bronchi may be readily observed in the fresh specimen with the naked eye. In the respiratory tract of the pig, following the dissecting away of the lung substance, the slitting open of the airway, and the careful sponging away of mucus, the elastic membrane, characteristically creamy white in color, may be seen shining through the epithelial layer. In the trachea, by close examination, the interwoven appearance of the fibers is evident, the membrane showing as a plexiform mass or network, with meshes running in a longitudinal direction. In the larger bronchi the appearance is that of longitudinal whitish-yellow streaks, raised into folds, where the elastic fibers are concentrated into dense strands or trabeculae, as many writers have pointed out. As the limbs of the tree are followed out to the smaller branches, the fibers of elastic tissue become less and less conspicuous.

APPEARANCE OF THE FRESH ISOLATED MEMBRANE

In the fresh lung of the pig the operation was tried of stripping off the elastic membrane entire. After clearing away the lung tissue and opening the main tubes, a beginning was made at the upper end of the trachea. It was found to be possible to dissect away, with the aid of forceps and a small scalpel, the mucosa containing the elastic membrane as its densest constituent. The stripping of the trachea in this manner was quite easily accomplished. As progress was made down the bronchi, however, difficulties were encountered and lacerations of the membrane were frequent. The main bronchial stems were followed, the membrane being cut across at the smaller branches. It was found to be quite a difficult task to make a clean stripping of the elastic membrane in the medium-sized bronchi, and in the smaller ones it was impossible, since the membrane became very thin and delicate. However, enough of the system was obtained in this way to make a good preparation.

When spread out there appeared a Y-shaped band of elastic tissue, the diverging limbs of the fork rapidly diminishing in width and density, and showing perforations for the branches

of the bronchi, as well as a ragged appearance along the edges. The tracheal portion was practically intact. When stretched and allowed to snap back, this membrane acted, as one would expect, much like a rubber band. Before the dissection it was noted that the trachea and bronchi, when pulled upon, showed the usual smart recoil. After the elastic membrane had been removed, however, the recoil was very sluggish indeed, and might hardly be said to exist. This demonstrated quite conclusively that the elastic membrane is by far the most important element in the recoil mechanism of the respiratory tract.

STAINED AND CLEARED WHOLE MOUNTS

The forked elastic strap, obtained as above described, was wrapped loosely around a small shell vial, tied, and fixed in 10 per cent neutral formalin for twenty four hours. It was then washed, dehydrated, and stained in toto in Weigert's elastic tissue stain. Then, after alcoholic extraction and clearing in benzine and oil of wintergreen, the specimen was studied by the binocular and compound microscopes.

The elastic membrane as thus prepared was found to be much too dense for the study of the individual elastic strands, which, to be made visible, had to be spread apart somewhat with the aid of dissecting needles. Especially was this true when the specimen was to be examined with the compound microscope. Often a slender piece had to be dissected off and teased carefully with needles in oil of wintergreen before the individual fibers could be plainly seen, so numerous and closely approximated were they.

Examinations of preparations made in this way disclosed the following facts regarding the elastic fibers. They run, as the authors have pointed out, in a longitudinal direction, i.e., parallel to the axis of the tube in the wall of which they appear. Each fiber follows a gently undulating course, and they branch frequently, so that there is formed in this way a meshwork of elastic fibers, the meshes being elongated longitudinally. The fibers are of different sizes, the average being much larger than that of those found immediately beneath the epithelial layer. The

largest fibers are quite equal to, if not greater than, the largest of the adventitia fibers. Their branching is after the manner typical of elastic fibers, there being a sharp angle where the branch is given off. The meshwork which they form is very close and dense. Where branching of the bronchus occurs, the membrane may be followed uninterruptedly into the diverging limbs, and, while upon the saddle-shaped ridge at the apex of the acute angle formed by the bronchial branches the fibers are not so characteristically longitudinal, yet they take this form immediately below the fork. There is a tendency for the fibers to be arranged in fasciculi, which themselves interbranch. In the trachea the fasciculi are flattened and the branching is more frequent, whereas in the bronchi, especially in the larger ones, the fasciculi are stouter and run for considerable distances without branching. Here, too, the entire membrane is especially dense, the fasciculi being very conspicuous.

Thus the elastic membrane forms a complete extensible sheath for the epithelium of the respiratory tract. If we were to dissolve away all but the elastic membrane from the bronchial tree, its form would still be perfectly represented. It is a veritable arbor elastica.

Small strips similarly prepared from the respiratory tract of the sheep and dog show the same general condition.

APPEARANCE IN CROSS-SECTION

Illustrations of cross-sections of the elastic membrane of the respiratory tract and descriptions based on examinations of cross-sections are to be found in the literature, and I shall here give only a brief account of a typical specimen. A fresh sheep's lung, together with the trachea, was obtained and fixed in formalin. The lung tissue was then dissected away, exposing the bronchial tree, which was photographed. The specimen was then washed, dehydrated, and stained en masse with Weigert's elastic tissue stain. After extraction and clearing with benzine and oil of wintergreen, pieces of trachea and left main bronchus were cut out, carefully numbered, and embedded in paraffin, the situations of the blocks thus obtained being marked upon the photograph of

the entire specimen. Thus the exact location of each section is known. Sections were cut from these blocks and either mounted as they were or counterstained. In this way sections were obtained of representative regions of the entire airway.

The general appearance of the elastic membrane, in cross-section, is much the same throughout the entire respiratory tract. The low power shows a sharply marked dark blue ring in the deeper layer of the tunica propria, which is resolved, by the higher powers, into a ring of thickly set blue dots—the cut ends of the elastic fibers. This ring of fibers is very conspicuous indeed in these preparations. By focusing up and down, branchings can be made out. The fiber-ends are of different sizes and often they occur in close groups; these groups, together with single strands, being collected into fasciculi, as seen in the whole mounts. In the larger bronchi this grouping and fasciculus formation is especially conspicuous and the fibers are very large. The longitudinal folds present in the mucosa of the bronchi in some animals were practically non-existent in my specimen of sheep's bronchus.

From the main ring of longitudinally disposed fibers, in the larger tubes, a few small elastic fibers can be seen to spring medially, and to be disposed in a loose, plexiform manner immediately beneath the epithelium, in the thin inner layer of the tunica propria. These fibers are much finer than the average size of those of the elastic membrane, and run in various directions, many of them circularly. Luciani ('11) and others mention them. They seem to serve the purpose of maintaining an elastic contact between the elastic membrane and the epithelial layer so that the latter will quickly and easily follow the movements of the former. A certain amount of freedom of the epithelial layer upon the elastic membrane is allowed.

Laterally, too, fibers are given off from the elastic membrane which pass out to mingle with the fibers of the adventitia. Some are connected loosely with the elastic tissue of the perichondrium surrounding the cartilages and others are interwoven with the muscle fibers. These adventitia elastic fibers form a loose felt-work, the strands running in various directions, but the impression is that most of them follow the direction of the bronchi with which they

are associated. They are not closely packed, like the fibers of the elastic membrane, nor do they make up, collectively, nearly such a mass of elastic material. Thus their action in shortening the respiratory tract, in the bronchi at least, must be very meager compared with that of the elastic membrane. In the lower end of the trachea, however, they were quite abundant. Connection of the fibers of the elastic membrane with the cartilages, muscle, and connective tissue of the outer layers, although loose and slight, yet probably makes for a smooth and orderly shortening of all layers of the respiratory tract during the contraction of the elastic layer.

As the elastic tube is followed down the bronchial tree, it is seen to diminish in density with its reduction in cross-section, and the fibers become of smaller average size, but even when the smallest bronchi are reached it is still a distinct layer. Indeed, it is here quite conspicuous, its main features—position, longitudinal direction, and closeness of apposition of fibers, etc.—being the same as in the larger bronchi. In the bronchioles the elastic membrane forms a conspicuous sheet underlying the muscle. Here, too, a large number of fibers lie outside of the muscle layer, and many of them run longitudinally; these fibers are often connected with the elastic membrane. As the bronchiole is followed down, the elastic membrane is seen to become gradually thinner and its terminations are in connection with the elastic tissue of the walls of the terminal air chambers, as many authors point out (Foster, '93, p. 436, et al.).

DISCUSSION

From a study of the structure of the bronchial tree; two facts emerge with clearness: that this branched system of tubes is *extensible*, and that it is *capable of recoiling* to its original condition.

That it is extensible is plainly deducible because longitudinal elastic fibers are such a prominent part of it, and because the tissues holding together the cartilages are of a loose character and are capable of being stretched to some extent. More convincing still is the experimental evidence; we are able to stretch the isolated tubes of the system in a fresh preparation. Every

branch of the tree is capable of being drawn out, and when they are all stretched the volume enclosed by the boundary marked out by their terminal points is much greater than that enclosed by these points when the branches have contracted. This, it is plain, is a phenomenon inextricably bound up with the expansion of the lung; in fact, it is one of the most outstanding features of this expansion.

In expansion of the lung, then, the bronchi stretch. In incomplete inspiration, of course, they may not all do so; some portions of the lung, particularly in the upper lobes, as Keith ('09) has pointed out, may be unexpanded in ordinary quiet breathing, and in individuals leading a sedentary life these regions may be practically unused. But in the healthy lung, in full inspiration, all the limbs of the bronchial tree are stretched, and, as we shall see, the trunk of the tree—the trachea—is also stretched.

If a demonstration were needed to convince us that there must be a stretching of the bronchial tree in inspiration, it would be afforded by a comparison of the levels marked out by the lower margin of the human lung in full inspiration and in full expiration. As every clinician knows, there is an interval of considerable magnitude here. Writing on the human lung, Norris and Landis ('17, p. 101) state that the "respiratory displacement of the lung is most marked in the axillary line" and that "during forced breathing the excursion may amount to 9 cm. ($3\frac{1}{2}$ inches)." The amount of the descent of the diaphragm, particularly in forced breathing, is evidence of the same kind. Starling ('20, p. 1091) states that this excursion, at the domes, in quiet breathing averages about half an inch, and Norris and Landis ('17, p. 101) give the corresponding figure as three-quarters of an inch. The latter authors state that in forced respiration the interval may amount to $2\frac{1}{2}$ inches, and even as much as 5 inches. Such facts can mean only that the lung is stretched along its sagittal axis during inspiration, since there is but slight, if any, downward movement of the apical region during the inspiratory phase.¹ Some writers consider

¹ Keith ('09, p. 187) says: "During inspiration the apical resonance does not extend into the neck, but decreases (Colbeck), and by placing a tambour on the neck over the apex of the lung it is found that the apex descends whenever the diaphragm is well in action, even if the first rib remains stationary."

that there is even at the apex some slight inspiratory expansion upward (Norris and Landis, '17, p. 97) as well as outward and forward. Thus the piston-like action of the diaphragm stretches the entire lung upon its long axis. The lung is stretched also, of course, anteriorly and laterally. The useful diagrams of Keith (Hutchison, '09, pp. 196-198) show graphically all these movements. Such a marked stretching of the lung substance can only occur with a stretching of the bronchial tree, for such a result would be impossible if it were inextensible. Hutchison ('09, p. 180) believes that the trachea and large bronchi stretch during inspiration.

In this connection Miller's ('21) results are interesting. This investigator made casts of the main bronchi and branches of the lower right lobes of the lungs of two dogs of equal size and weight, one lung being in the condition of full expansion and the other of total collapse, and found upon comparing them that both the main stem bronchus and the branches were longer in the expanded lung. While not positive proof of extension of the tubes, since the comparison could not be made of the collapsed and filled conditions in the same lung, and since a totally collapsed lung was used (instead of one in the condition of moderate distension normal for the expiratory phase) for comparison with the expanded one, yet the results go far to convince one of an elongation of the bronchial tree in inspiration. It may be remarked here, too, that Miller's (21) studies upon the muscle bands in the bronchi and bronchioli lead him to conclude that (p. 703) "When the action of these bands of muscle is studied it will be found that the arrangement is such that provision is made for the elongation of the bronchi and bronchioli during inspiration and the subsequent shortening during expiration."

It is evident, then, that the bronchi and bronchioli are stretched during inspiration, and particularly during forced inspiration. But there is, also in forced inspiration at least, a stretching of the trachea. Keith ('09) has emphasized the fact that the root of the lung, together with the heart, undergoes a descent with inspiration accompanying the descent of the diaphragm, and Macleod ('20), following Keith, has this to say (p. 343): "The

root of the lung, which has generally been regarded as more or less fixed, undergoes in normal breathing a definite forward, downward and outward movement, and the heart shares in this movement (Keith)." Others have noted the same phenomenon, vide Groedel's ('13) diagram on page 206 of Norris and Landis (17). X-ray study of the diaphragm movements shows that the central portion has an up-and-down excursion, and Macleod ('20, p. 338) states that "The central portion of the diaphragm does not move much in normal respiration, but in forced respiration its movements may be considerable." Thus the lower end of the trachea descends with deep inspiration, and the tube is stretched. The stretching of the entire airway in full inspiration is in keeping with the essential unity of its system of elastic fibers, and particularly those of the elastic membrane. The fact that the bronchus enters the hilus of the lung very obliquely favors its free movement here.

To realize the importance of the stretching of the bronchial tree, it is only necessary to imagine it to be perfectly inextensible. It seems obvious that then the free expansion of the lung tissue would be impossible.

The stretching of the bronchial tree bears some resemblance to the stretching of a rubber band where one end is fixed and the other pulled upon. The movement of different points upon the band becomes greater and greater as we progress from the fixed end to the end pulled upon; so, too, the movements of points situated at different levels of the respiratory tract are least in the region of the larynx and greatest at the termination of the system.

From what has already been said, it is apparent that the free expansion of the upper end of the lung is largely dependent upon the downward movement of the lower end, for the upper portion cannot expand downward until the lower part has made way for it. The upwardly directed bronchial radicles must have room to elongate. True, the upper end can expand outward and forward, and there may be, as we have said, some slight upward expansion, but its filling would be greatly impaired if the downward movement should be impeded. Since this downward stretching of the lung is dependent largely upon the flexibility

of the root region, it follows that anything which interferes with the freedom of movement here will interfere with the free filling of the lung, and particularly with the upper end of it. Is it not possible that the fibrotic changes following inflammatory conditions might cause such an inhibition of normal movement in the lung roots? In this connection Keith ('09, p. 189) remarks as follows: "In cases where the roots of the lungs are bound to the posterior or stationary wall of the thorax through adhesions set up by mediastinitis, Wenckebach observed that both the respiratory and circulatory movements were abnormal in character." We may well ask what would be the results due to the tissue changes consequent upon chronic tuberculous lymphadenitis in the tracheal and bronchial lymph nodes. If such changes tended to fix the roots of the lungs, or to interfere seriously with the extensibility of the bronchi, grave impairment to lung expansion would result, and this impairment would be particularly in the upper lobe. Is it not possible that the greater frequency of tuberculosis in the upper lobes of the lungs than in the lower is due to the crippling of the expansion in the upper lobe consequent upon inhibition or elimination of the normal movements of extension and contraction in the trachea and main bronchi? One cannot conceive how a stiffening of any important part of the bronchial tree could be without its damaging effects upon the function of the area of lung supplied by it.

THE RECOIL MECHANISM

It is apparent to anyone who makes a careful study of the elastic membrane of the trachea and bronchi that we have in it to deal with the densest aggregation of elastic fibers in the whole respiratory system below the larynx. In comparison with this membrane, the density of the remaining elastic tissue of the airway and that surrounding the terminal respiratory chambers is relatively slight. This, together with the further facts that the fibers run parallel with the various limbs of the bronchial tree, as well as with its trunk, and that they are attached above to the relatively fixed larynx, forces upon us the conclusion that we have here to do with a very important, if not the main, element in the

elastic recoil mechanism of the lung. It is quite obvious, too, that to insure an efficient retraction of the entire lung substance, this retraction should be centered along the axes of the lung substance, viz., along the bronchi. Immediately upon the relaxation of the inspiratory musculature of the thorax there is a recoil of the elastic tissue of the entire lung substance. There is a shortening of the entire bronchial tree, together with a diminution in the space within the respiratory tissue proper. This even shortening of the bronchi prevents bending or buckling of the tubes, with resulting partial or complete obliteration of the lumen, which would occur if the lung tissue should recoil without a coincident retraction of the limbs of the bronchial tree. It is by a combination of the recoil of the respiratory portion of the lung, of the pleura, and of the airway proper that the air is driven out in a smooth and rapid manner, without the trapping of air due to wrinkles or folds occurring in the bronchi. The entire system of elastic tissue works together, and the action of one part cannot be considered without coordinating it with that of the remainder.

The situation of this elastic membrane—in the tunica propria close to the epithelium—is most favorable for the insuring of a smooth mucosa. Luciani ('11, p. 404), Foster ('93, p. 435), and others mention this function of the elastic membrane, particularly for the trachea.² The loose connection of the membrane with the epithelium through the finer network of fibers in the vascular and adenoid layer enables the epithelial layer to follow the movements of the membrane without being torn or wrinkled. Were it placed between the cartilages of the airway, its total length would be enormously reduced, and its efficiency correspondingly decreased; then, too, transverse folding of the mucosa, and perhaps tearing of it, would be apt to occur. Still more unfavorable, perhaps, would be a situation farther out beyond the cartilage zone—for then would occur an inevitable partial or

² The continuity of the fibers of the elastic membrane above with the abundant elastic tissue of the larynx permits of stretching of the trachea in upward movements of the larynx, as in swallowing or bending the head backward, and of returning to the normal position (Hutchinson, '09, et al.).

complete obliteration of the lumen, not only due to the forcing into it of the cartilages of the walls, with the contraction of the elastic fibers, but also on account of transverse folding of the epithelial layer. Situated as it is, the mucous membrane is able to follow the extension and contraction of the airway without injury and without offering any impediment to the airflow.

BLOOD VESSELS AND OTHER STRUCTURES

It seems apparent that the blood vessels which accompany the bronchi should be capable of stretching, or at least of straightening out, and of returning to the unextended condition, to follow the movements of the bronchial tree. It is not unlikely, too, that they should show some difference in respect to the arrangement of their elastic tissue in comparison with other vessels of the body which are not forced to undergo like deformation, and this point is being investigated. It is doubtful, however, if we have in the vessel walls any appreciable part of the recoil mechanism of the lung. Similarly, the structure of the lymphatics and nerves must permit of their being stretched.

OSCILLATORY MOVEMENTS OF THE BRONCHI?

Keith ('09), Miller ('21), and others have favored the view of a widening and narrowing movement in respiration, which is said to occur in the angles or spaces between diverging bronchial branches. In inspiration the branches which center at a common point separate from one another, like (as Keith puts it, p. 184) "the opening of a Japanese fan." This conception seems to rest upon the idea that the lung tissue enclosed by two or more convergent bronchial limbs expands, thus forcing them apart, and so the available space is increased.

There are some difficulties in the way of too liberal an acceptance of this notion. The bronchial tree, for instance, is not built quite like a Japanese fan. In opening the fan the lateral ribs must swing through a very wide arc, and such an extensive swaying movement would be impossible for the bronchi. Moreover, the idea fails to take into account the fact that these bronchi which are supposed so to move apart are surrounded on all sides by

expanding lung tissue (assuming expansion to be normal and full), and that the tendency toward widening of the angles caused by the expansion of the tissue between the limbs would be largely, if not altogether, neutralized by the expansion of the tissue on the opposite sides of the limbs. Such a movement, if it did occur, might widen the bronchial tree, but could not lengthen it, and so would not account for the downward stretching of the lung in inspiration.

Miller's ('21) efforts in search of the truth in this connection are open to criticism. He describes and shows us figures of two casts of the bronchial trees from the lower right lobes of the lungs of two dogs of equal size and weight. Both specimens, however, were made from lungs removed from the pleural cavity, and accordingly cannot be relied upon as picturing exactly normal conditions. These casts Miller seems to look upon as representing the contrasted forms of the bronchial tree in expiration and in inspiration. Upon comparing them, it is obvious that in the cast said to show the expiratory phase there has been an approximation of the bronchial radicles, but serious objection may be raised against this cast, since it was made from a lung in a condition of total collapse—a condition which is hardly identical with the phase of expiration. The cast purporting to exhibit the phase of inspiration, too, is open to objection, since the lung from which it was made was expanded under artificial conditions. We do not know whether or not the form taken by this bronchial tree is the same as that which would have been taken by it in its normal environment, in the phase of full inspiration. Thus Miller's demonstration does not prove the point which he claims for it.

It behooves us to keep an open mind regarding this question, however, lacking convincing demonstration in favor of either side, and when one looks at a cast of the bronchial tree and realizes that the bronchial limbs point in general toward the regions of greatest expansile movement, one is inclined to admit that there is something in the idea of bronchial inspiratory divergence. However, with the idea before us of an extensible bronchial tree, it is not necessary to place so much dependence upon this Japanese-fan concept to explain lung expansion, and, in any event, such a

movement under existing conditions, would be absolutely inadequate to account for lung expansion and contraction without having associated with it an extension of the limbs of the bronchial tree.

One should note, too, with Keith ('09) that the expansile movements of the lung in the transverse plane are practically limited to the outward and forward (and intervening) directions, there being no opportunity for the lung to expand backward, and but little or none inward. Even when we allow for the in-drawing of the mediastinal walls, due to the elongation of their contents, which follows the descent of the central part of the diaphragm (Keith, '09) in vigorous inspiration, it is plain that the main directions of expansion of the lung, in the transverse plane, are as Keith has pointed out. It follows, then, that if the tissue lying between the main bronchial stem and the posterior and medial surfaces is to expand, it must be by the shifting outward of this main bronchial stem. This would mean some slight divergence of the two main bronchial stems from one another.

SUMMARY

1. The conspicuous network of longitudinal elastic fibers which forms a membrane in the tunica propria of the entire bronchial tree of mammals, and which I have referred to as the "elastic membrane of the bronchial tree," may be dissected off en masse from the fresh trachea and larger bronchi, and the branched strap so formed behaves when stretched much like an elastic band.

2. The specimens so obtained may be stained, cleared and mounted for study.

3. This membrane, when considered as an isolated unit, is in general form a replica of the entire bronchial tree, and its density corresponds fairly directly with the size of the tube in the wall of which it is found. It becomes thin and delicate in structure toward the ends of the bronchioles, and is connected with the elastic tissue of the respiratory portion of the lung. It constitutes a unified system of branched elastic tubes which extends from the larynx to the outermost limits of the air channels.

4. Each of these tubes represents an axis for the extension and contraction of the lung substance, and in the expansion

and recoil of this substance the movements follow these axial lines. The recoil of the lung substance, considered collectively, is thus a summation of the recoil of its various elements, centered along these bronchial axes. The structural unity of the system emphasizes its functional unity.

5. Evidence is reviewed to show that the bronchial tree stretches in inspiration and contracts in expiration. The actual excursion of any point within the bronchial tree is greatest at the extremities of the limbs and least at the upper end of the trachea. The tips of the caudally directed twigs of the lower lobe show the greatest excursion. The amount of stretching, of course, varies with the depth of the inspiration.

6. The filling of an area of lung tissue with air is associated with a stretching of the air-carrying tubes supplying it. With a rigid bronchial tree the movements of expansion and contraction of the lung, as we know them, would be impossible.

7. The elastic membrane is by far the densest aggregation of elastic fibers in the entire bronchial tree, and this fact, together with the consideration that its fibers run parallel with the axes of the various branches of the tree, and that the bronchial tree, deprived of this membrane, suffers a loss of most of its ability to recoil, forces us to the conclusion that in this membrane we have by far the most efficient part of the recoil mechanism of the entire bronchial tree. When, further, the relative amount of the elastic tissue in this membrane is compared with that in the remainder of the lung substance, we realize that we have in this system of elastic tubes a very important, if not the most important, portion of the recoil mechanism of the entire lung.

8. The conception of a stretching and contracting of the bronchial tree in respiration minimizes the importance of the conception of an oscillatory movement on the part of the bronchial branches, after the manner of the ribs of a fan. It seems quite possible that the main bronchial stems may shift forward and outward in inspiration, thus providing for the expansion of the vertebral and mediastinal region of the lung tissue.

9. Much of the expansion of the upper lobe is secondary to the downward shifting of the entire lung, and it follows that

freedom of movement in the lung root is essential to freedom of movement of the lung, particularly in the upper lobe. Thus, pathological conditions which tend to immobilize the roots of the lungs (such as tuberculosis of the neighboring lymph glands and associated adhesions) will tend to cripple the movements in the upper lobes, and so may help to account for the selective affinity of this region of the lung substance for the tuberculous process. More widely, anything which acts to stiffen the limbs of the bronchial tree will affect adversely the function of the part supplied by these limbs, and so will tend to precipitate pathological processes therein.

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The reaction of living cells in the tadpole's tail toward
starch, agar-agar, gelatin, and gum arabic.

Small droplets of corn- or arrowroot starch were injected into the transparent tails of frog larvae. Uncooked, semicooked, and completely cooked starch were studied. The results were studied in the living animal, and also after staining with iodine. Uncooked starch causes a mild attraction for leucocytes, which phagocytize the granules and retain them unchanged as cell inclusions indefinitely. Boiled starch exerts a moderate attraction for leucocytes; it is quickly transformed by substances both outside and inside the leucocytes into colorless dextrin or sugar. Semicooked starch granules exert a most intense attraction on leucocytes, which surround the granules, phagocytize the smaller ones, and, in the course of a few hours, transform them into substances which do not stain with iodine. Agar-agar, gelatin, and gum arabic, introduced in capillary glass tubes, exert an attraction on leucocytes similar in kind and intensity to that exerted by semicooked starch. The leucocytes approach and phagocytize them rapidly. The other tissue cells show no response toward starch or the other substances used.

THE REACTION OF LIVING CELLS IN THE TADPOLE'S TAIL TOWARD STARCH, AGAR-AGAR, GELATIN, AND GUM ARABIC

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SIX FIGURES

During the past twelve years the authors have studied the character, mode of growth, and behavior of living cells in the transparent fin expansion of the tails of amphibian larvae. By means of chloretone anesthesia and an upright glass chamber, or microaquarium (Clark, '12), it has been possible to see individual cells and tissues with great clearness and in their normal location, to follow the same cells for hours, days, and even weeks, and to watch their growth and their response to various stimuli. The cell types especially studied have been connective-tissue cells, wandering cells, and blood-vessel and lymphatic endothelium. The response of these cells toward a number of injected substances has been described in previous communications.

The success of the injection experiments is largely dependent upon the use of substances which are readily injectable through fine glass cannulae, thus producing a minimum of injury, which will remain unabsorbed long enough to allow time enough for an observable reaction on the part of the cells and tissues, and which will be visible. Suspensions of granules, such as carmine and carbon, fulfill these conditions and have been used in experiments of this kind. Oily substances also lend themselves to such experiments and their effect has also been studied. Small globules of an inert oil (paraffin oil), nutritive oils (olive-oil globules and emulsions of yolk of egg and cream), and an injurious oil (croton oil) are the substances of this category which have been used in previous experiments.

Substances which may be dissolved or suspended in paraffin oil can be used for such experiments, since former observations showed that paraffin-oil globules are inert when inserted into the tails of tadpoles. With other more easily diffusible substances it is possible to fill a small glass cannula (measuring 10 to 20 μ at the tip) with the substance to be tested, to insert it in the fin in the desired position, and then to break off the end, leaving a small glass tube open at both ends inside the fin. The epidermis heals over the opening within a few minutes. This method is not satisfactory, however, unless the substance contained in the tube is either stained or mixed with some visible material.

In the first series of experiments reported here, starch, in small enough quantities to permit of distinguishing individual granules, was injected through fine glass cannulae into the transparent tail fins of living tadpoles. Later, the effect of other substances of somewhat similar physical constitution, such as agar-agar, gum arabic, gelatin, etc., was studied.

EXPERIMENTS WITH PLAIN GLASS TUBES

Since it was found impracticable to inject uncooked starch grains, agar, celloidin, gum arabic, and gelatin on account of the great difficulty encountered in trying to force these substances out of the tips of fine cannulae, these materials were drawn up into small glass needles which were then inserted in the fin, the end broken off, and the tube tucked under the skin in the desired position. The glass tubes then served as small test-tubes, open at both ends, through which the enclosed substance could slowly diffuse into the surrounding tissue.

Control experiments were performed with plain glass tubes—empty except for the surrounding medium of sterile chloretone solution—which were inserted in the manner described above. By a study of the reaction produced by these tubes it was possible to distinguish between the response of the living cells to the various substances which we wished to test and the reaction to the mere presence of a foreign body.

The presence of such a glass tube served as a stimulus for a rather severe epidermal reaction which took place soon after its

insertion. The epidermal cells became opaque and pigmented and had the appearance of being heaped up on that part of the surface immediately over the ends of the tube. This reaction of the epidermal cells was more marked when longer tubes were used than in the case of the very short ones. It subsided during the first twenty-four hours after the operation.

Near-by wandering cells approached the tube and flattened out on its surface and, an hour or more after the insertion of such an empty glass tube, a slight stickiness of leucocytes to the wall of a few blood vessels in the vicinity was observed. A few leucocytes were seen to migrate from these vessels toward the tube. During the next two days the leucocytes and wandering cells lined up along the tube, forming a single layer of flattened cells which completely surrounded it. After the formation of such a cellular layer, the supernumerary leucocytes wandered away from the region. Such tubes were observed for periods of two to four weeks, and, after the formation of this leucocytic layer, they apparently exerted no influence on the surrounding cells and tissues.

The insertion of plain glass tubes into the tadpole's tail evidently produces a pronounced but transitory reaction of the epidermal cells of the region. Their presence also attracts leucocytes from near-by blood vessels to a slight degree. In other words, these tubes produce a mild form of foreign-body reaction.

THE REACTION OF LIVING CELLS TO STARCH GRANULES

Corn-starch and arrowroot were the two kinds of starch used. They were injected in three different forms: 1) as uncooked starch, 2) as cooked starch, and, 3) as semicooked starch (cooked just to the point of gelatinization). The uncooked starch was treated with ether, which was allowed to evaporate, and the starch then suspended in sterile distilled water. The cooked starch was prepared by mixing starch with distilled water and boiling for ten to fifteen minutes. 'Semicooked' starch was made by mixing the starch with sterile distilled water and heating in a water-bath to the point of gelatinization.

In the case of the two varieties of cooked starch, it was possible to inject small quantities of the paste into the tail fins by means of the small glass cannulae. With the uncooked starch, however, the individual grains are too resistant to permit of their being forced through the small cannulae, consequently small glass tubes containing uncooked starch grains were inserted under the skin in the manner just described.

The experiments were performed on larvae of *Rana pipiens*, *Rana catesbiana*, and *Bufo lentiginosus* Fowleri.

Chloretone anesthesia and the same methods of injection and observation described in previous articles were employed (E. R. Clark, '12, '16).

After observing the cells in the vicinity of the injected starch for varying intervals of time, the tadpoles were fixed in tincture of iodine (in 70 per cent alcohol). The tails were then cut off and cleared in graded strengths of glycerin.

The microscopic appearance of the three forms of starch is different. The uncooked starch grains are round refractile bodies with sharp, regular outlines. The grains of cooked starch are translucent and irregular in shape. The semicooked granules are opaque and rounded. In the preparation of both cooked and semicooked starch, many of the granules swell and burst, discharging their contents into the surrounding medium, leaving only the shells of the former granule.

I. EXPERIMENTS WITH UNCOOKED STARCH

In a number of tadpoles, a glass tube containing uncooked starch was inserted in one fin and a similar tube containing cooked starch in the other. Wandering cells from the tissues and leucocytes from the blood vessels were attracted toward both tubes, but they migrated in much greater numbers toward the tube containing the cooked starch.

On the morning following the experiment, many leucocytes were present around both glass tubes, but those around the tube of cooked starch were much more numerous. Leucocytes were packed in the lumen of the latter tube throughout its extent, and all of the starch was apparently inside of leucocytes. A few

leucocytes were also present inside the tube of uncooked starch, but the greater part of the lumen was still filled with the unchanged refractile starch grains.

On the second day after the insertion of the tubes the central portion of the tube of cooked starch was empty, while leucocytes, granular in appearance, were still numerous around the ends of the tube. The tube of uncooked starch still contained many free granules. A number of leucocytes containing refractile starch grains were found at the ends of the tube, in the surrounding tissue spaces, and inside of near-by lymph vessels.

The glass tubes were usually extruded from the tail after a week or ten days. In the case of the tubes of uncooked starch, a number of leucocytes containing refractile starch grains were left behind in the tail fin. During the subsequent days many of these leucocytes wandered away from the injection site, some of them entering lymph vessels. However, a number still remained near the original site of the tube, and these were followed for several days and, in one specimen, for as long as a month with no change in the appearance of the starch grains inside of leucocytes. Uncooked starch grains, therefore, acted apparently like foreign bodies, such as minute drops of paraffin oil or particles of carmine and carbon. Wandering cells and a number of leucocytes from the blood vessels were attracted toward the uncooked starch and they proceeded to ingest the starch grains, which then remained as cell inclusions for practically indefinite periods of time. It is probable that the cellulose covering of the uncooked granules prevents action on the granule by the ferments in the leucocytes and tissue fluid.

II. EXPERIMENTS WITH COOKED STARCH

When boiled starch paste was injected into the tail fins of tadpoles, the starch granules appeared as translucent bodies with irregular pliable-appearing outlines. These starch grains disintegrated rapidly and disappeared after fifteen to forty-five minutes (depending upon the quantity injected). In one case only three granules of cooked arrowroot were injected in one region. These three were watched carefully. At ten minutes after

the injection, all three granules were mere shadowy outlines and sixteen minutes after the injection they had disappeared altogether. The tadpole was immersed immediately in a weak solution of iodine and then cleared in glycerine. Microscopic examination disclosed a faint bluish tinge at the site of injection. In this case the starch grains had dissolved without any visible reaction on the part of the surrounding tissue cells and before any wandering cells or leucocytes had reached them.

In other cases, in which a larger quantity of cooked starch was injected, some grains were still present a half-hour after injection and leucocytes and wandering cells moved toward the injected starch and engulfed the undissolved portions of the starch grains. Such a specimen when treated with iodine one-half hour after injection showed two large blue granules with leucocytes adherent to them, a scattering of finely granular almost diffuse blue stain, and several leucocytes with a bluish tinge in their cytoplasm. Tadpoles, fixed in iodine, one hour or more after injection of thoroughly cooked starch, showed no trace of blue color.

These experiments, as well as those just described in which the starch was introduced into the fin inside of glass tubes, showed that cooked starch is a stronger chemotactic stimulant for leucocytes than uncooked starch. They also showed that the tissue fluid itself possesses some substance capable of dissolving and digesting cooked starch grains.

No cells except leucocytes were affected in any visible way by the presence of the starch.

III. EXPERIMENTS WITH SEMICOOKED STARCH

Starch (in the form of corn-starch or arrowroot) was mixed with sterile distilled water and heated over a water-bath just to the point of gelatinization. In this case the starch grains are pliable enough to permit of injection through a fine glass cannula, yet substantial enough to remain as discrete rounded bodies which do not dissolve in the tissue fluid. These semi-cooked starch grains are rather opaque and easily distinguishable from the round refractile uncooked starch grains, as well as

from the translucent, irregularly shaped grains of cooked starch. Starch grains prepared in this way were injected into the tail fins, the region studied, and camera-lucida tracings made of the region near the injection.

The near-by connective-tissue cells, blood vessels, and lymphatics showed no reaction to the presence of the starch. Wandering cells, located near the injection site, showed an immediate response: they made their way directly toward the starch and their movement was very rapid.

Ten to fifteen minutes after injection, leucocytes were observed migrating from near-by blood vessels. In contrast to the experiments with croton oil, the leucocytes in this case came out of the vessels situated nearest the injected material, instead of those further away. These leucocytes moved swiftly toward the starch and, arriving at the site of injection, they proceeded to surround the individual starch granules. More leucocytes continued to come out of the blood vessels and to arrive upon the scene until, after a period of one to three hours (the length of time depending upon the amount of starch injected), no starch grains could be seen, the injection site being occupied by a dense mass of leucocytes (figs. 3 and 4).

The diapedesis of leucocytes started sooner than after the injection of any substance tried in our previous experiments; the number of leucocytes which came out of the vessels was very much greater than that attracted toward injected substances, such as powdered carbon or carmine, fats such as olive oil, cream, yolk of egg, or toward the area of inflammation produced by injected globules of croton oil, or by infections. And, too, the movement of the individual leucocytes and wandering cells toward the starch grains was more rapid than that observed in the case of these cells under any other circumstances.

The active migration of leucocytes toward the site of the injected starch continues for several hours after all of the starch has been taken up by cells (fig. 4). This attraction for leucocytes is much more conspicuous in the case of the semicooked starch than in that of either the cooked or the uncooked varieties. In several instances in which cooked starch was injected into

the fin, a few semicooked granules, easily detected by their more opaque appearance, chanced to be present in the midst of the translucent cooked ones. When the individual starch grains were numbered and watched constantly for several hours, it was observed that the first leucocytes which approached the injected material invariably moved first toward the semicooked starch grains, flattening out on the surface of the grains, frequently passing by cooked grains, and sometimes even moving between two cooked granules on their way to the semicooked ones. In one such case ten starch grains were injected, and of these four were of the opaque semicooked variety. These four were completely surrounded by a mass of migrated leucocytes before any of the cells had approached the more thoroughly cooked granules. In the meantime the cooked granules had begun to dissolve and, in many cases, they were shells or shadows. After the semicooked granules had been completely enveloped by dense masses of leucocytes, the next leucocytes to arrive approached the remnants of the cooked granules and proceeded to ingest them. In other cases an occasional uncooked starch grain, detected by its high degree of refractility, was found in the midst of the injected starch. Such granules were not ingested by leucocytes until all of the cooked starch had been disposed of (fig. 2).

After the leucocytes had taken up the starch, their cytoplasm had a peculiar swollen and granular appearance which distinguished them from the other wandering cells. Some of these leucocytes remained near the site of injection for as long as a week after the injection of the starch.

Fixed specimens of the tails of larvae injected with starch showed that both the polymorphonuclear and the mononuclear varieties of leucocytes were attracted toward the starch grains.

THE FATE OF INJECTED STARCH

It has been mentioned already that the uncooked starch remained unchanged for weeks after being taken up by leucocytes. The thoroughly cooked starch starts to dissolve very quickly, as evidenced by the finely granular blue stain visible in the

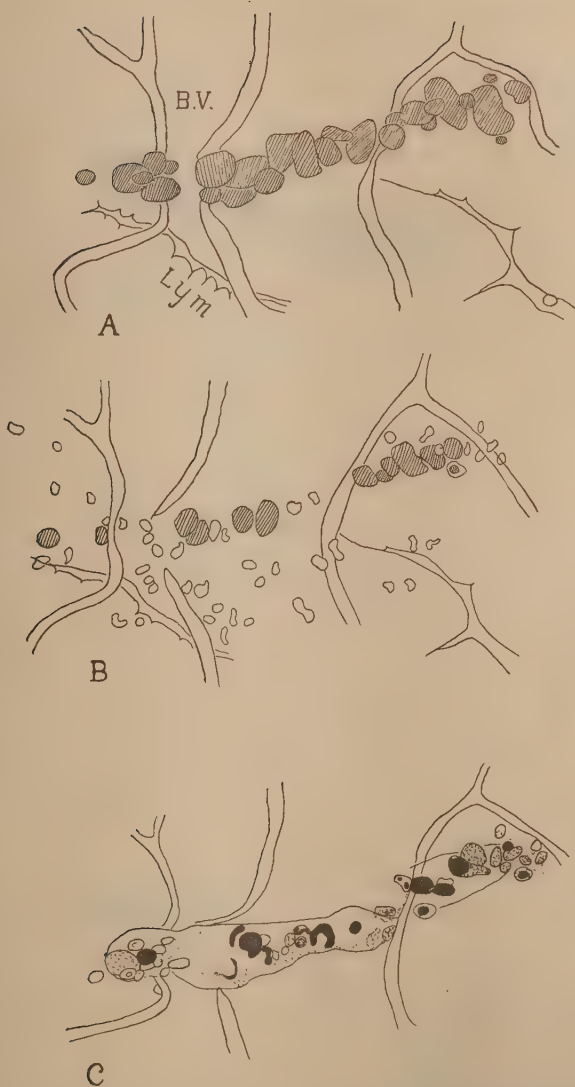


Fig. 1 A. Camera-lucida sketch of the ventral fin of a frog larva immediately after injection of semicooked arrowroot starch. The starch grains are shaded, leucocytes in outline. *Lym.*, lymphatic; *B.V.*, blood vessel. $\times 105$.

B. Camera-lucida sketch of the same region one hour later. Leucocytes are migrating toward the starch. Some of the starch has already been taken up by leucocytes. $\times 105$.

C. Same, after fixation in iodine. The boundary of the clear space around the injection area is indicated by a line. The portions stained blue with iodine are shown in solid black. The diffuse blue stain with iodine is indicated by dots. $\times 105$.

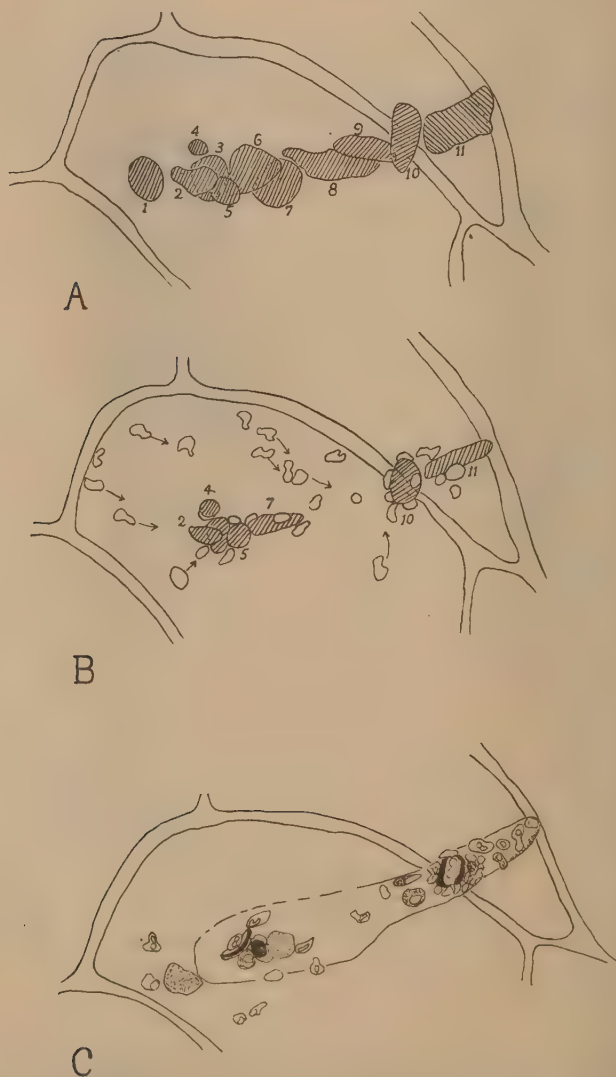


Fig. 2 A. Camera-lucida sketch of ventral fin immediately after injection of arrowroot paste. The starch grains are numbered. No. 4 is uncooked. Nos. 2, 3, 5, 10, and 11 are semicooked. Nos. 6, 8, and 9 are thoroughly cooked. $\times 123$.

area of injection, after fixation in iodine at intervals of ten to forty-five minutes after injection.

In the case of the semicooked starch, the starch is apparently converted into a soluble form inside of leucocytes. A series of tadpoles injected with semicooked starch was fixed at different intervals in iodine and then cleared in glycerin. In such a specimen in which only a few starch grains were injected, all of the grains were observed in the living animal to be inside of leucocytes at the end of an hour. After staining with iodine, an hour after injection, a few portions of starch grains showed up as dark bluish-black masses inside of leucocytes. In the cases in which uncooked granules were included in the mass of injected starch these, of course, showed up as blue-black bodies with regular outlines. Still other leucocytes were found with a diffuse blue stain in their cytoplasm. In addition, there were a number of leucocytes collected near the injection site and on their way toward it which were stained a yellowish brown similar to the rest of the cells in the fin. In all specimens fixed an hour to an hour and a half after the injection, a clear space was present which surrounded the area of injection and which contained no connective-tissue cells (figs. 1C and 2C).

In tadpoles, fixed at three hours after the injection of semicooked starch, a number of leucocytes could be found containing a definite blue tinge in their cytoplasm. The blue-black starch grains or parts of grains found in the one-hour specimens

B. Camera-lucida sketch of the same region forty-five minutes after the injection, showing leucocytes migrating toward the starch. The cooked starch grains 6, 8, and 9 have dissolved. Leucocytes have surrounded the semicooked starch grains and others are rapidly approaching them. $\times 123$.

C. The same region fixed in iodine one and one-half hours after the injection. Before fixation all of the remaining starch grains had been taken up or surrounded by leucocytes. No. 4, the uncooked starch grain, was inside a leucocyte which had moved it to a position between Nos. 2 and 3. The place of granule No. 10 was occupied by a clump of leucocytes. The iodine fixation shows No. 4 (uncooked) inside a leucocyte, distinct and unchanged in form. Parts of grains Nos. 3 and 10 appear as blue bodies surrounded by leucocytes. The other grains have been dissolved and many of the leucocytes show a bluish tinge in their cytoplasm (black dots in sketch). Some of the leucocytes are definitely polymorphonuclear, others mononuclear. The clear space around the injection area shows in this specimen as in figure 1, C. $\times 123$.



Fig. 3 A. Camera-lucida sketch of ventral fin, twenty-eight minutes after injection of starch (arrowroot). Grains Nos. 3 and 5 were uncooked, the others semicooked. A number of leucocytes have already reached granule No. 8, the others are approaching the injection site. $\times 105$.

B. The same region three hours after the injection. Numerous leucocytes present in the injection area and others migrating toward the former site of the starch. The starch grains have all been taken up by leucocytes. Grains Nos. 3 and 5 still show plainly as round refractile bodies. Clumps of granular leucocytes occupy the former sites of the other starch grains. $\times 105$.

C. Same region after fixation in iodine. Nos. 3 and 5 show as dark blue bodies unchanged in shape (black in sketch). Parts of Nos. 1, 2, 6, and 7 also stain blue with iodine. Several leucocytes near the former site of No. 8 have a bluish tinge (dots in sketch). No clear area at this stage. $\times 105$.

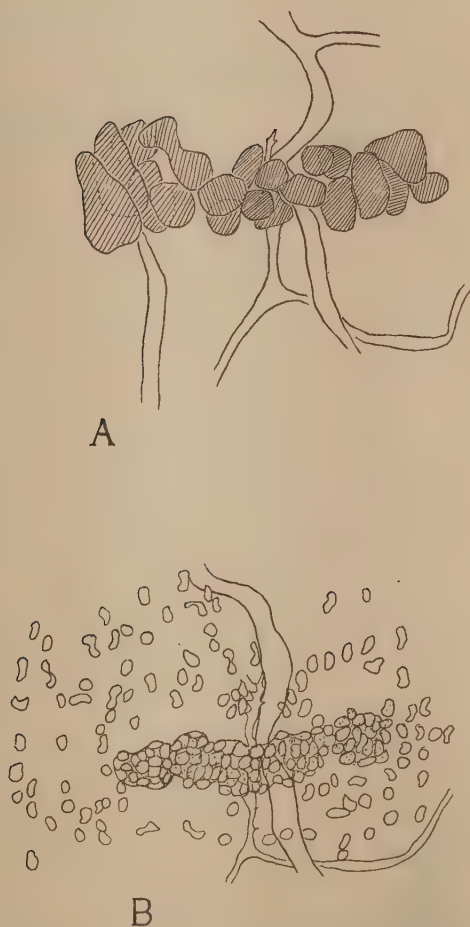


Fig. 4 A. Camera-lucida sketch of area immediately after injection of arrowroot starch.

B. The same region six hours later, showing violence of leucocytic reaction caused by the presence of semicooked starch. $\times 123$.

were not seen here except of course in those specimens in which uncooked starch grains had been injected along with the others. The clear space around the injected area was absent in specimens fixed three hours after injection (fig. 3C).

In larvae fixed in iodine at five to six hours after injection of semicooked starch, the blue had entirely disappeared (with the same exception of the uncooked starch grains). The mass of leucocytes at the former site of the starch granules contained no trace of blue, but was dark brown. However, when these cells were examined with high magnification the brown color was found to be confined chiefly to the nucleus, the cytoplasm being a pale yellow and there was no noticeable color difference between the cells collected near the injection site and the other wandering cells, while the brown color was not nearly so intense as that found in the muscles.

The iodine reaction showed that the semicooked starch is taken up by leucocytes by which it is dissolved (the diffuse blue stain) and that after three hours it is changed into another substance. Since the characteristic dextrin and glycogen reactions with iodine were not obtained, it is probable that the starch is changed into a colorless dextrin or sugar. Attempts were made to trace by microchemical tests this further process in the digestion of starch, after the blue color with iodine could no longer be seen. The osazone (phenylhydrazine) test for sugar and the test with Benedict's solution (copper sulphate and sodium hydroxide) were tried with specimens in which starch had been injected one to two hours previously, but always with negative results. Control specimens in which a solution of dextrose was injected into the fin also yielded negative results, although the same solution showed the characteristic reactions on a glass slide. We were unsuccessful in our attempts to detect sugar in the tail fin by microchemical means.

Some experiments were performed in order to compare the results obtained in these experiments with those obtained by treating such starch solutions with *ptyalin*.

Arrowroot paste, cooked to the point of gelatinization, was treated with iodine and examined on a glass slide under the

microscope. The starch grains were all blue-black in color and in addition a bluish fluid with fine blue granules was present between the starch grains.

Such starch paste, treated for two to three minutes with ptyalin (saliva) and then fixed in iodine showed some blue-black granules, a greater amount of the finely granular blue material, and, in addition, a few irregular deposits of a pale, yellowish-brown color.

After eight minutes, treatment with iodine showed a number of pale blue, and a still greater number of purple and lavender granules. In addition, more of the large irregularly shaped yellowish-brown masses were present, and also a great many small brown granules, smaller than the original starch grains. After still longer treatment with ptyalin—fifteen to twenty minutes—followed by staining with iodine, no blue staining material remained, and only the large yellow-brown masses and the small brown bodies were present.

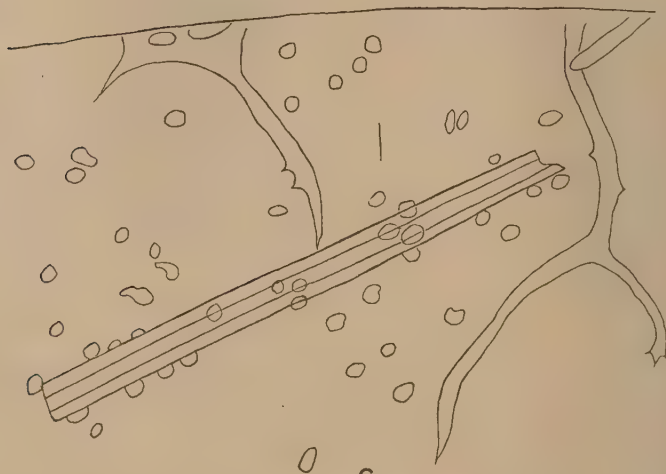
In comparing the fate of starch injected into the subcutaneous region of amphibian larvae with the fate of starch treated with saliva, it will be seen that, in the former case, the starch is changed more slowly than in the latter, and that, after being acted upon, it is apparently changed not into a dextrin or glycogen which stains brown with iodine, but into sugar or dextrin which does not stain with iodine.

REACTION OF LIVING CELLS TO TUBES CONTAINING AGAR-AGAR, GELATIN, AND GUM ARABIC

In connection with the reaction of leucocytes to starch granules observations on certain other substances are of interest. These substances were enclosed in minute glass tubes and inserted in the tails of tadpoles by the method described earlier, with the object of discovering a suitable medium for testing easily diffusible substances whereby they might be localized and their too rapid diffusion prevented. Since agar-agar is digested and absorbed to only a slight degree in the alimentary tract, it seemed probable that this would prove a suitable material for this purpose such as paraffin oil proved to be in the case of substances of an oily nature.



5



6

Fig. 5 Camera-lucida drawing of the region near a glass tube containing agar-agar, nineteen hours after inserting it in the dorsal fin. Sketch shows large numbers of leucocytes which have surrounded the tube which have collected in great numbers at the two ends, and which are still migrating in large numbers from the near-by vessels. $\times 123$.

Fig. 6 Camera-lucida drawing of the region near a glass tube, empty except for a suspension of Berlin-blue granules in distilled water, seventeen hours after inserting it in the dorsal fin. Note the difference in the number of leucocytes attracted toward this glass tube compared with that attracted toward the tube containing agar-agar as shown in figure 5. $\times 123$.

The tubes were made, as described, in the form of small glass cannulae measuring 15 to 20 μ at the tip. The agar was dissolved in boiling distilled water, sucked up into the cannulae while still liquid, and allowed to solidify. A thin layer of agar remained adherent to the outside of the tube. Such a cannula containing agar-agar was inserted in the desired position in the fin, then the tip was broken off and tucked under the skin with the aid of a sharp needle. The hole made in the epidermis healed over within five to ten minutes after the operation. Immediately after the insertion of such a tube the tadpole was transferred to the observation chamber and records made of the region involved.

As might be expected, the epidermal reaction, described in the experiments with the plain glass tubes, took place also in this instance.

Near-by wandering cells moved rapidly toward the tubes of agar. Within ten to twenty minutes after the insertion of the tube, leucocytes began to stick to the endothelium of near-by blood vessels and to migrate through the walls. The leucocytes moved toward the tube and flattened out on its surface, collecting in especially large numbers around the two ends of the tube (fig. 5). Leucocytes continued to adhere to the blood-vessel endothelium, to pass through the walls, and to migrate toward the tube for more than twenty-four hours after the insertion of the tube. After the first two days the migration of leucocytes from the blood vessels ceased, but the leucocytes, both clear and pigmented, still remained flattened against the sides of the tube and heaped around the ends. Three or four days after the beginning of the experiment, the lumen of the tubes was filled with leucocytes which had acquired a granular appearance, apparently as the result of ingesting the agar. Such tubes remained inside the tail for periods of three weeks and over, and at the end of this time the tubes were still filled with the granular leucocytes and many cells were still adherent to the sides of the tube. By this time the rest of the migrated leucocytes had wandered away from the region of the experiment.

Tubes containing substances of somewhat similar constitution were inserted into the tail fins of tadpoles. These were gelatin and gum arabic. They were prepared in the same way as was the agar-agar; that is, they were dissolved in boiling distilled water, sucked into the cannula while still hot, and allowed to cool before the cannula was inserted into the tail.

With both these substances results were obtained similar to those described in the case of agar-agar. There occurred an epidermal reaction, which was particularly marked over the ends of the tube, and which later subsided, a rapid and intense response of near-by wandering cells, sticking of leucocytes to the walls of near-by blood vessels very soon after the insertion of the tube, migration of leucocytes rapidly and in large numbers toward the tube, and collection of these cells around the tube, particularly at the open ends, followed by migration of leucocytes into the tube and eventual phagocytosis of the substances contained therein. This intense leucocytic reaction is shown in figure 5.

From these results it seems evident that such substances as agar-agar, gum arabic, and gelatin act as powerful stimuli for the migration of leucocytes. The leucocytic reaction called forth by these substances is similar in rapidity and intensity to that caused by the injection of semicooked starch grains. As in the case of the starch, the presence of these substances occasioned no response on the part of connective-tissue cells or of the endothelial cells of blood vessels or lymphatics other than the change in the blood vascular endothelium disclosed by the adherence of leucocytes to the vessel walls.¹

¹ Since the completion of these experiments the work of Wolf ('21) has been published. This investigator made use of salt-free water solutions of agar as a carrying medium for testing the effect of a large number of chemicals on leucocytes in vitro. She found that only 4 per cent of the leucocytes used in the experiment were attracted toward the salt-free agar alone. We are unable to state whether the difference in these results is due to the different type of animals used in the two experiments (rabbits and dogs in Wolf's experiment), or to differences in the behavior of leucocytes in vitro compared to those found in the living animal, or to some differences in the preparation of the agar used in the two sets of experiments.

DISCUSSION

It seems unnecessary at this time to refer to the numerous investigations which have been made upon the attraction of leucocytes toward different substances. Gabritchewsky ('90) classified various chemical substances as positively chemotactic, negatively chemotactic, and indifferent in their effect on leucocytes. Wells ('18) and his pupils, in recent studies, have tested many drugs in vitro and have added a long list to these three classes. Recent authors attribute the difference in the attraction of different drugs for leucocytes to surface tension phenomena: positively chemotactic substances being those which lower the surface tension of the cell, causing the cytoplasm to flow toward them. Moreover, Fenn ('21) considers phagocytosis to be a surface-tension phenomenon. In his study of the reaction of leucocytes to quartz and carbon particles, he found that the carbon was phagocytized three or four times as rapidly as the quartz, and he attributed the difference in rate to the more unstable condition of the carbon suspensions.

With the exception of the studies of Metchnikoff on the phagocytosis of carmine in the transparent tails of amphibian larvae, all these studies on the reaction of living leucocytes to different chemical substances have been made in vitro.

All of our observations on the reactions of cells to different substances injected into the transparent tails of tadpoles have been made in vivo on cells in their natural environment. Our earlier studies have shown that the different cell types respond in different ways to the various substances introduced into the fin. For example, lymphatic endothelium reacts to fatty substances by growing toward them, but not to starch grains; connective-tissue cells will take up carbon and carmine granules, but not starch grains or fat droplets.

With regard to the attraction of leucocytes toward the various substances used in our different experiments, we have noticed great differences in the degree of response.

Thus, paraffin oil proved to be practically an indifferent substance, since the reaction shown by the few wandering cells present near the site of injection was mild and transitory and no

greater than that produced by the simple insertion of the injection needle.

Next to paraffin oil may be placed foreign particles such as carbon or carmine granules and uncooked starch grains, which attracted wandering cells and, an hour or so later, leucocytes from near-by blood vessels in moderate quantities, which migrated toward the injected granules and proceeded to phagocytize them. These particles remained inside the cells indefinitely. The reaction which occurred after the introduction of small glass tubes, empty except for the surrounding chloretone solution, was essentially similar.

Fatty substances, such as small globules of olive oil, and oleic acid and emulsions of cream and yolk of egg, attracted leucocytes in larger numbers. The injected fat was taken up rapidly by the leucocytes and conveyed to near-by lymphatics, into which it was discharged in a soluble form.

In the case of inflammations produced by the injection of small globules of croton oil, leucocytes migrated from the blood vessels at some distance from the site of injury in greater numbers than in the case of the other substances just mentioned. The leucocytes did not phagocytize the croton oil, but became stationary at some distance from the injected globule, sending out processes until they came to resemble small fibroblasts. After the extrusion of the croton oil, they rounded up and later wandered away.

The most intense leucocytic reaction yet obtained was that produced by injecting starch granules, in a semicooked gelatinous form, and by the introduction of agar-agar, gum arabic, and gelatin, enclosed in small glass tubes. Leucocytes began to migrate through the walls of near-by blood vessels ten minutes after the insertion of these substances into the fin and their movement was very rapid. The leucocytes phagocytized the starch grains, while in the case of agar-agar, etc., they migrated into the ends of the tubes and eventually ingested all of the substances inside.

SUMMARY

Starch grains introduced into the transparent fin expansion of the tadpole's tail produce different reactions according to the state of the starch, that is, according to whether it is uncooked, partially cooked, or fully cooked.

Uncooked starch granules attract a few leucocytes, which collect about the starch granules, and if the granules are not too large, phagocytize them. Such granules remain unchanged as cell inclusions, inside the leucocytes, for an indefinite period of time. The resistance to digestion of uncooked granules is probably due to their cellulose covering.

Cooked starch, in which all the granules have swollen and burst, so that most of the starch is outside the shells of the granules, dissolves rapidly, even before the arrival of many leucocytes, and undergoes a change into some other substance which does not stain with iodine. There is a moderate attraction for leucocytes, which evidently aid in the transformation of the starch. It is apparent that there are enzymes both outside and inside the leucocytes which act upon the cooked starch.

Semicooked starch granules exert an extreme attraction on leucocytes, which migrate rapidly and in large numbers toward such granules, completely surround them, engulfing them if small, and transform them, within the space of a few hours, into a substance which does not stain with iodine.

Agar-agar, gum arabic, and gelatin, enclosed in small glass tubes and inserted in the tail fin, provoke a reaction on the part of the leucocytes similar in kind and intensity to that produced by the semicooked starch granules.

No visible reaction on the part of the other subcutaneous tissues present in the vicinity of the injected substances—blood-vascular and lymphatic endothelium, nerves, and connective tissue—was observed in response to the presence of any of these substances.

It is a pleasure to have this opportunity to express to the authorities of the Marine Biological Laboratory, Woods Hole,

Massachusetts, where a part of this work was done, our appreciation of the facilities of the laboratory which were so generously granted.

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New York.

On the idiosome, Golgi apparatus, and acrosome in the
male germ cells.

An attempt has been made to synthesize the results of recent investigations on the relation of the Golgi apparatus and idiosome to each other and to the acrosome in developing sperm cells. The idiosome in spermatocytes was originally defined by Meves on the basis of its supposed relation to the centrioles, whence arose the common confusion of the idiosome with a true 'attraction sphere.' It now appears that the idiosomic material is closely related to another cytoplasmic element, the Golgi apparatus. The latter may assume several appearances depending on the technique employed, but its condition in the living cell is probably that of a number of discrete rodlets. These may be clustered with the idiosomic material around the centrioles, or may be scattered through the cytoplasm without reference to the cell centers, in which case the idiosomic material is similarly distributed—a small mass to each Golgi element. The idiosome is thus to be defined by its relation to the Golgi apparatus rather than the centrioles. In the maturation divisions the Golgi elements are distributed to the spermatids by a complex dictyokinesis, and the idiosomic material probably accompanies the Golgi pieces in this distribution. In the spermatids the idiosome and Golgi apparatus may be reconstituted into a compact group (the familiar 'sphere' complex of many authors) or may remain in a scattered condition. The acrosome forms as a special differentiation product of this idiosome-Golgi complex, the exact method being characterized by great variation in detail in different animals, but fundamental similarity in essentials.

ON THE IDIOSOME, GOLGI APPARATUS, AND ACROSOME IN THE MALE GERM CELLS

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THREE FIGURES

The terms idiosome, Golgi apparatus, and acrosome have been rather generally applied in recent years to three characteristic structures of the male germ cells of animals, where they have proved a source of error and confusion for practically all students of spermiogenesis. Indeed, our understanding of these structures is now so beclouded that, even in recent text-books of cytology, they have usually been dismissed with a few casual comments, for the most part incorrect. The one point upon which there seems now to be fairly general agreement is that these three cytoplasmic components are definite and distinct parts of the cell, unrelated, in any immediate way at least, to mitochondria, chromidia, or other formed elements of the cytoplasm. In other words, the structures considered in this paper are to be thought of as parts of the cell comparable from the standpoint of morphological identity with the mitochondria, the centrioles, and perhaps even the nucleus. Recent studies have made it increasingly clear that the chief source of past errors is to be found in the technical methods which were formerly employed almost exclusively for the study of spermatogenesis. But since the problems of the cytoplasm have been attacked with a better appreciation of the fundamental difficulties involved, there has been an immediate clarification of many disputed points. My own interests happen to have centered in this field, and as a result of studies on the formation of the sperm in a variety of animals I have arrived at a fairly clear idea of what seem to me the fundamental features in the morphology of the idiosome, Golgi apparatus,

and acrosome in the male germ cells. I propose, therefore, in this paper to attempt a synthesis of my own and others' results, with a view to putting the available facts into a connected story which may serve as a supplementary chapter to the current text-book descriptions of spermiogenesis. That this account will prove to be correct in all respects is rather improbable, for our knowledge of the facts in different animal groups is still fragmentary and in many cases quite unsatisfactory; but I do hope to arrive at some scheme, satisfactory at least in a descriptive way, which may serve as a point of departure for further researches. No attempt will be made to treat the subject from an historical standpoint except in the interests of clearness, and illustrations will be drawn wherever possible from my own material, with which I am most familiar. The account will be limited to the conditions found in *typical, flagellate sperms*, since these are the only ones so far adequately investigated. I shall endeavor, also, to bring out particularly those features which still require further research for their elucidation.

THE SPERMATOCYTES¹

When the testis of almost any animal (with the exception of many insects)—a mollusc or salamander, for example—is fixed in one of the orthodox cytological fluids, such as strong Flemming, and stained with Fe-hematoxylin and a counter-stain in accordance with the usual procedure, there will be found in the cytosome of each spermatocyte² a roughly spherical mass which takes the stain somewhat more darkly than the surrounding cytoplasm. Under good conditions, it is possible to demonstrate the centrioles within this mass, located centrally and usually in the form of paired granules (fig. 1A). To this spherical body Meves, in 1896, gave the name of *idiozom*,³ a

¹ The acrosome, being characteristic of the developing sperm, is not of course present as such in the spermatocyte stages. This section deals, therefore, only with the idiosome and the Golgi apparatus.

² Primary spermatocytes are referred to unless specifically stated to the contrary.

³ The usual English spelling is *idiozome*.

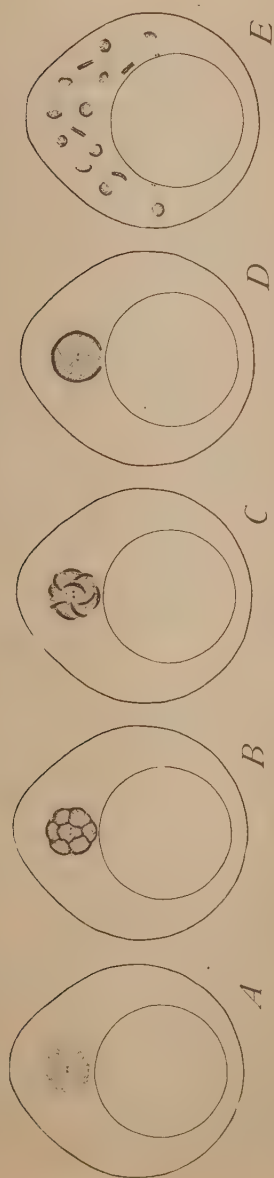


Fig. 1 Diagram to illustrate the structure of the idiosome and Golgi apparatus in the primary spermatocyte as demonstrated by various technical methods. Only the outlines of the nucleus and cytosome are indicated. The idiosome is stippled, and the centrioles are represented as simple granules. *A*, after the customary fixation with fluids containing acetic acid; *B* and *D*, frequent appearance after treatment by the impregnation methods for demonstrating the Golgi apparatus; *C*, particularly after chromosome fixatives with acetic acid absent or present in very small amount; *E*, scattered condition of the idiosome and Golgi apparatus in many insects as demonstrated by many of the special technical methods for the Golgi apparatus.

term which was to stand "fuer die spezifisch beschaffene Huelle, welche die Centralkoerper in den maennlichen Samenzellen umgibt." Subsequently Meves ('03) suggested the substitution of *centrotheca*, a term which never succeeded in displacing its predecessor in general usage. More recently, Regaud ('10) has suggested the spelling *idiosome*, a change which has much to recommend it, and will be adopted in this paper. It is clear from Meves' remarks in adopting the term *centrotheca*, that he regarded the association of the idiosomic substance with the centrioles as the fact of primary importance—a view with which I cannot agree for reasons that will presently appear.

The real nature of the idiosome was from the very beginning obscure, and the fact that it was spherical and contained the centrioles immediately led to a most unfortunate confusion with the so-called *attraction sphere* of Van Beneden ('83) and the *archoplasm* of Boveri ('88). These latter terms were applied by their sponsors to the unusual protoplasmic differentiations which arise in connection with the centrioles during the division of certain animal eggs, but which, as has been repeatedly urged by various workers (for example, Duesberg, '20), have nothing whatever to do with the idiosome. The grounds for this distinction will appear more clearly in subsequent paragraphs. The point to be noted is that the terms *archoplasm* and *attraction sphere* have a very special and limited application, if indeed they have any very good basis in fact at all, and in any event are not to be used as indicative of any direct relationship with the idiosome.

This very simple condition of the idiosome⁴ and its surroundings is characteristic of material which has been fixed in fluids containing a considerable amount of acetic acid. If the same kind of material (mollusc or salamander) be fixed in fluids in which acetic acid is absent or present only in small amount,

⁴I have not thought it worth while to include a discussion of the internal structural features which have been occasionally described in the idiosome. The meaning of these, and particularly their homologies, is as yet by no means clear. The reader who is interested in these features is referred to the papers of Papanicolaou and Stockard ('19) and Gatenby and Woodger ('21) on the idiosome of the guinea-pig.

such as Benda's modified Flemming or Flemming without acetic, the results are rather strikingly different. In a spermatocyte (stained with Fe-hematoxylin) from such a preparation (fig. 1C), the idiosome itself appears much as before, but closely applied to its periphery appear a number of small, crescentic rods which stain very sharply. These rods have of course long been known under a great variety of names (Archoplasmaschleifen, Centra kapsel, Pseudochromosomen, formazioni periidiozomiche, Nebenkern, etc.), since in certain animals—the molluscs particularly—they are often preserved in more or less perfect condition even after fixation in strong Flemming. They are, as Gatenby and others have recently shown, the representatives of the Golgi apparatus in the spermatocyte. The term apparatus is a very unhappy one, for in this case, as in so many others, the Golgi material apparently forms no connected *apparatus* at all. It would be in the interests of clarity to refer to this substance simply as the Golgi rods or some equivalent descriptive expression. It is important to note that these rods are actually separate units, and one does not get the impression that they are interconnected in any way by secondary fusions.

Again, in material prepared by one of the impregnation methods, for example, the silver reduction techniques of Golgi or Cajal, the idiosome and its surroundings may assume a somewhat different aspect. The idiosome substance itself may be more or less invisible, but its position is clearly marked by the Golgi apparatus which encloses it. The material of the Golgi apparatus is blackened intensely in successful preparations and has sometimes been described as forming a true network or reticulum, comparable to the classical figures of the '*apparato reticolare interno*' (fig. 1B). This appearance of the Golgi apparatus has been described by numerous authors as characteristic of many somatic cells after impregnation by silver nitrate or osmic acid, but the occurrence of the Golgi material in reticular form has rarely been described in spermatocytes. Perroncito ('10) thus describes and figures the Golgi apparatus in *Paludina* (a mollusc); but it should be noted that Gatenby

('18) has figured the Golgi apparatus in the same animal as made up of separate rodlets as in figure 1C.

Finally, after the same impregnation methods referred to in the preceding case, the Golgi apparatus may appear as a shell, closely applied to the idiosome and often (always?) incomplete on the side adjacent to the nucleus (fig. 1D). The shell itself may be more or less solid, or may appear as though made up "of a number of filaments of varying thickness." Duesberg ('20) has described such a structure for the Golgi apparatus in the spermatocytes of the opossum, and I have found more or less similar conditions in the salamander after silver nitrate (Cajal) impregnation. Furthermore, following osmic acid impregnation, I have found (salamander) that the 'shell' is sometimes entirely disrupted and the fragments scattered about in the cytoplasm in the vicinity of the idiosome.

How are these conflicting appearances of the Golgi apparatus to be harmonized; or, indeed, is there any reason to believe that the Golgi apparatus is always developed in the spermatocytes on the same structural plan? Unfortunately, we are not in position to give a categorical answer to these questions, chiefly because the majority of workers have so far been content to study the Golgi apparatus by very limited technical means. It is accordingly difficult to say in any particular case whether the results are descriptively correct or mere technical distortions of the truth. However, my own experience leads me to believe that the discrepancies in the published accounts of the Golgi apparatus, in the spermatocytes at least, are largely traceable to the untrustworthy results of the admittedly capricious impregnation methods (silver and osmic acid). Gatenby's work on *Paludina* and my own results on *Plethodon* (a salamander) seem to me to indicate unmistakably that the probable source of our difficulties is to be accounted for along the following lines. When the material is preserved in one of the chrome-osmic mixtures, which are generally agreed to be among the best-known cytoplasmic fixatives, the condition of the Golgi apparatus is almost invariably that of a cluster of rodlets applied to the surface of the idiosome (fig. 1C). After a

silver-nitrate impregnation the Golgi rods may undergo a slight disintegration resulting in their running together to form an apparent network or reticulum (fig. 1B), while still further disintegration or 'smearing' of the impregnation deposits may transform the Golgi apparatus into a more or less complete 'shell' (fig. 1D), or even disrupt the rods completely and scatter the fragments in the neighboring cytoplasm. Finally, after fixation in acetic-acid mixtures, the entire Golgi apparatus may be completely disintegrated and dissolved (fig. 1A). That the actual condition of the Golgi apparatus in the living spermatocyte is most nearly comparable to a group of *separate rodlets* is strongly supported not only by the general appearances which accompany what cytologists call 'good fixation,' but by the additional fact that fragmentary observations on living material seem to point in the same direction.⁵

It is clear from what has now been stated that the idiosome and the Golgi apparatus form in many spermatocytes a topographical unit, the center of which is occupied by the centrioles. Two questions at once suggest themselves: 1) is the close relation of the idiosomic substance and Golgi material merely a chance coincidence of location and, 2) what relation, if any, exists between these two materials and the centrioles, which would justify the retention of Meves' original definition of an idiosome and the consequent confusion with the attraction sphere and archoplasm?

It is possible to give at least a partial answer to these questions by reason of the unique conditions which obtain in many (perhaps all) insects. In the insects the idiosome seems sometimes to occur as a compact body in the very early spermatocytes,

⁵ I wish to state specifically that this attempted explanation of the various appearances of the Golgi apparatus in the spermatocytes after different types of technique is *not* to be extended inconsiderately to conditions in somatic cells as well, in which the Golgi apparatus is most commonly described as having the form of a reticulum or network. The facts now known about the possible effects of technical treatment ought, however, to lead to a more critical scrutiny of the actual conditions in cells of all kinds. The facts suggest that in tissue cells, whose tenure of life is presumably a long one, the Golgi rods normally undergo more or less fusion resulting in the reticular condition so frequently described.

but eventually (in all known cases) and frequently (the Hemiptera furnish particularly good examples) from the very earliest spermatocyte stages onwards, the idiosome is represented only by many *separate* masses of idiosomic material each accompanied by a Golgi rodlet, the whole constituting a so-called Golgi body (Bowen, '20). These are at first definitely concentrated toward one pole of the cell and are thus directly comparable to the compact idiosome already considered. We are obviously dealing with an idiosome the constituent parts of which have become separated from one another. In the growth period the Golgi bodies begin to migrate away from their definitely polar position (fig. 1E), and eventually become scattered throughout the cytoplasm as I have described fully in the Pentatomidae (Bowen, '20). However, at no time is there any indication of a separation of the Golgi rodlets from the masses of idiosomic substance.

The behavior of the centrioles during these same stages offers a number of points of interest. In the very early primary spermatocytes the behavior of the centriole is fully known in comparatively few forms, but presumably the centriole from each spindle pole of the last spermatogonial division is always carried over directly into the succeeding primary spermatocyte. In the mollusc and salamander this centriole soon divides, but the parts remain close together until the maturation prophase are well advanced, and are located, as noted above, in the idiosome. In the Hemiptera, however, the centrioles behave very differently, for after dividing, the daughter centrioles begin at once to migrate around the nucleus (fig. 1E) until eventually they occupy diametrically opposite positions on the nuclear membrane. Meanwhile they each divide precociously in anticipation of the two rapidly succeeding maturation divisions. Throughout the migration of the centrioles and until the divisions of maturation begin, the Golgi bodies maintain no striking topographical relations to the centrioles.

The conditions in the insects furnish, it seems to me, an excellent basis for the interpretation of the idiosome structures as a whole. We are to think of the Golgi bodies (Golgi rodlet

plus idiosomic fragment) as the units of idiosome construction. These bodies tend, for some unknown reason, to collect in the vicinity of the centrioles, just as other parts of the cell (mitochondria, for example) may be polarized toward the centrioles when these bodies are together. If the centrioles remain thus together, the Golgi bodies likewise remain massed around them, giving rise to the familiar idiosome and Golgi apparatus complex of many authors. If, however, the centrioles separate, the polarization of the cell is apparently destroyed, and the idiosome fragments migrate out into the cytoplasm, together with the mitochondria, and become scattered throughout the cell in haphazard fashion. It becomes, therefore, perfectly clear that the important and primary relation is that which exists between the Golgi material and the idiosomic substance, while their joint relation to the centrioles is merely a topographic one. The basis for Meves' definition of the idiosome thus breaks down entirely and it is evident that we must look for a rigorous definition of the idiosome in terms of its interrelation to the Golgi apparatus rather than to the centrioles. I have suggested elsewhere (Bowen, '20) that this relation may possibly be of a permanent nature, the two components forming essentially a unit cell structure. Finally, this interpretation clears up much of the current misconception which obtains concerning the protoplasmic differentiations that sometimes surround the centrioles, such as the archoplasm, attraction sphere, centrosome, etc. These differentiations (so far as they have any existence in fact) have presumably a true organic relationship to the cellular centers, being connected with the processes of spindle formation, while the relation of the idiosomic substance to the centrioles is purely topographical and frequently does not obtain at all. Certain aspects of this interpretation will be further clarified by comparison with the following sections.

THE MATURATION DIVISIONS

It is not my intention in this paper to go extensively into the phenomena exhibited by the idiosome and Golgi apparatus during the maturation divisions, in part because the details

are as yet insufficiently known, but chiefly because the earlier workers almost invariably failed to observe any of the details by reason of the technique employed, and there is, accordingly, little to confuse the reader in the literature so far published. I wish, however, to indicate certain broad features of the division phenomena as an introduction to the spermatid stages, which have long been a source of inextricable confusion, due largely to ignorance of the fate of the idiosome and Golgi apparatus during the division phases.

From the facts now available it appears that there are two (possibly three) methods of distributing the Golgi apparatus in the spermatocyte divisions, the method being perhaps correlated with the previous condition of the idiosome. In the case of the idiosome which early loses its compact form (fig. 1E), the separate Golgi bodies undergo more or less fragmentation, prior to mitosis, which involves both the Golgi rods and the idiosomic substance. The resulting fragments (so-called dictyosomes) gradually collect around the poles of the spindle during the metaphase, each pole receiving an approximately equal quantity (Bowen, '20). During the telophase, when the centrioles are separating in preparation for the second maturation division, the dictyosomes are dispersed throughout the cell and are again accumulated around the spindle poles at the subsequent metaphase. During the telophase they may again become more or less scattered in the cytoplasm.

In the case of the idiosome which retains its compact form until immediately before the maturation divisions (figs. 1A to D), the end result is similar, but the intermediate steps are somewhat different. In this type, immediately preceding division, the idiosome and Golgi rodlets are divided into two groups which migrate with the centrioles to the spindle poles, where they are found at the metaphase just as in the preceding case (see, for example, Ludford and Gatenby, '21). Subsequently they become scattered throughout the cytoplasm and in the secondary spermatocytes are again assembled around the centrioles in compact form. The process is repeated in the second maturation division.

It is possible that a third type of division occurs in which the collection of the Golgi fragments around the poles is omitted, as Duesberg ('20) has described in the opossum. But the evidence for this is very scanty and further research may prove it quite erroneous.

In this brief outline of the division phenomena, I have purposely omitted mention of several important points which are still unsettled. The first of these involves the possible fragmentation of the Golgi rodlets prior to their accumulation around the centrioles at metaphase. A breaking-up or division of the Golgi rodlets certainly occurs in the Hemiptera which I have studied, and I have thought that there was good evidence of the same thing, in the salamander. Gatenby, on the other hand, insists that the Golgi rods are merely sorted out *intact*. The question is an exceedingly difficult one to settle on account of the inadequate technical methods now available, but it is one well worth further study. The second point in dispute has to do with the fate of the idiosomic substance during division. In the Hemiptera the dictyosomes are too small to analyze satisfactorily, and it is therefore impossible to say whether or not they retain the essential structure of the Golgi bodies through the division stages. From the fact that the idiosomic material can be found associated with the Golgi substance as soon as the dictyosomes have again fused to form aggregates sufficiently large to study accurately, I have concluded that in all probability each dictyosome is essentially a miniature Golgi body. Each Golgi fragment would thus be accompanied by a small mass of idiosomic material, and the distribution of both substances would accordingly be identical. No specific stain for the idiosomic material is at present known, and since it cannot be satisfactorily made out in small amounts, the question cannot be immediately solved. In the idiosome of the compact type, it seems certain that the substance accompanies the Golgi material to the spindle poles, but its subsequent fate is again obscured by the scattering of the Golgi pieces, and though it reappears with the latter around the centrioles in the late telophase, its exact intermediate behavior is uncertain. Ludford

and Gatenby ('21) believe that it becomes separated from the Golgi material in the interval—a view to which their figures do not lend particular support, and which to my mind runs counter to all the other available evidence.

It will be clear from the above that the exact behavior of the Golgi apparatus and the idiosome in the maturation divisions is very inadequately understood, but nevertheless that the Golgi material certainly, and the idiosomic material probably, is distributed to the resulting spermatids in a form identical, at least topographically, with the same materials of the original idiosome-Golgi complex of the spermatocytes. Furthermore, this opportunity may be taken to point out again that, as can be inferred from the above account, the idiosome has not been observed to take any part in the formation of spindle and asters during mitosis. This, I believe, is practically conclusive evidence that the idiosome is a cellular differentiation absolutely unrelated to attraction spheres or archoplasm, whose presumed function it is to provide material for the construction of the achromatic figure.

THE SPERMATIDS

At the close of the second maturation division the Golgi apparatus is present in each spermatid in the form of numerous, scattered pieces or dictyosomes. These may begin at once a process of fusion, so that the individual pieces become fewer in number, but larger in size. Presently they assume proportions not unlike the Golgi bodies of the growth period, and it is clear that each one is composed of the characteristic Golgi and idiosomic materials, arranged exactly as in the spermatocytes. According to the account of Ludford and Gatenby, the dictyosomes would perhaps undergo no fusion whatever, since they are, by description, complete Golgi bodies throughout the division stages. In any event, it is agreed that the Golgi apparatus and accompanying idiosome are usually present in the earliest spermatids in the form of more or less scattered Golgi bodies.

From this point on, however, the behavior of the Golgi bodies is subject to certain differences which may be conve-

niently classified under two characteristic types—types which recall the similar arrangements of the Golgi apparatus and the idiosomic material in the primary spermatocytes. These two types I propose to designate as follows: I, simple or fused type; II, compound or multiple type. These two types will be considered separately.

Type I, Simple or fused

In the great majority of animals thus far examined, the behavior of the Golgi bodies in the spermatid follows type I. In this type the Golgi bodies, formed as noted in the preceding paragraphs, gradually draw together, their idiosomic portions apparently fusing until eventually a compact mass is formed comparable to, and indeed directly homologous with, the characteristic idiosome of the primary spermatocytes as developed in the salamander and mollusc.

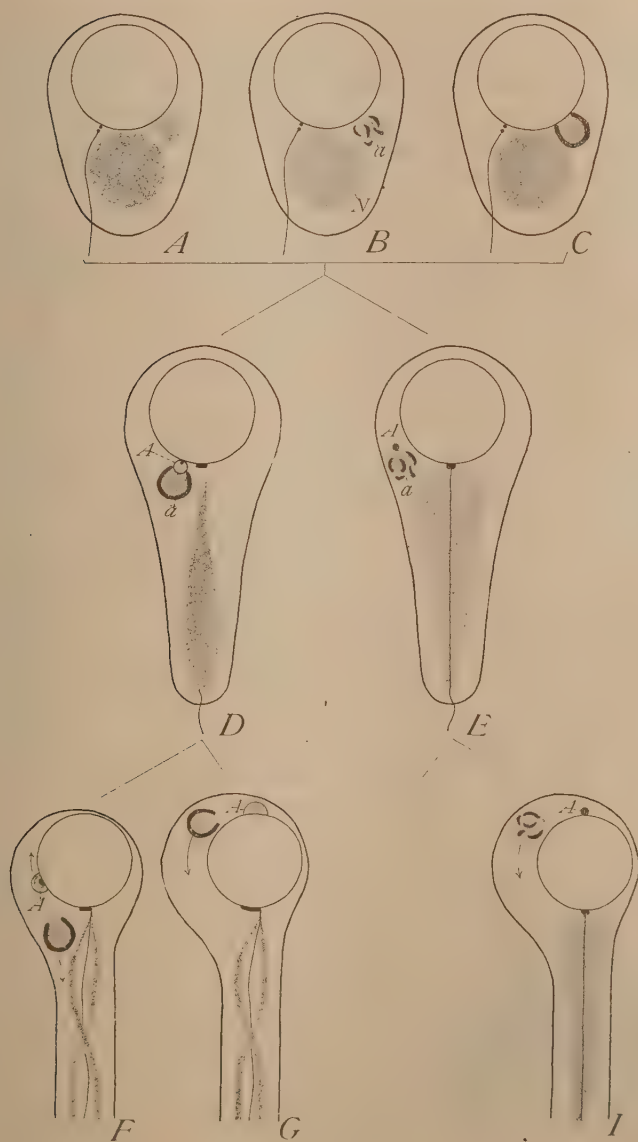
The mass thus formed may assume a variety of appearances exactly similar to those of figures 1A to D, and probably, in part at least, for the same reasons. Thus, in material fixed in acetic-acid mixtures the Golgi apparatus is completely dissolved, leaving only a spherical mass, the idiosome, or 'sphere' of many authors (fig. 2A). In material fixed especially in the chromosmium fluids with little or no acetic acid, the Golgi rodlets are sometimes clearly demonstrated, applied to the surface of the idiosome just as in the spermatocytes (fig. 2B). Very rarely a net-like Golgi apparatus comparable to that of figure 1B has been described (Perroncito, '10), but this is decidedly unusual. Very frequently, after impregnation methods, the Golgi material forms a solid shell enclosing the idiosomic substance, though here again the shell is incomplete on the side toward the nuclear membrane (fig. 2C). As a matter of fact, I have preparations of hemipteran testes in which, after the appropriate technique, all these various appearances (except the reticular condition of the Golgi apparatus) are clearly visible. The diagrams in figures 2A, B; and C are taken from these preparations. I had originally thought that the 'shell' condition shown in figure 2C represented the final step

in the fusion of the Golgi elements begun immediately after the second maturation division was completed. Recently, however, I have succeeded in demonstrating the presence (in *Euschistus*) of separate Golgi rods, as shown in figure 2*B*, so that the condition of figure 2*C* is perhaps to be explained as in part an artifact.

The Golgi apparatus-idiosome complex may be located in almost any part of the spermatid; but in insects, in which the mitochondria are aggregated into a solid, spherical mass or *nebenkern*, the idiosome is characteristically found near the angle between nucleus and *nebenkern*, and sometimes in a definite relation to the centrioles (figs. 2*A* to *C*). But the centrioles, it is almost universally agreed, are *never* located *within* the idiosome as is so characteristic of arrangements in the spermatocytes. Here, again, is a striking piece of evidence which tends to show that Meves' conception of the relation between idiosome and centrioles is erroneous, and that the relation between Golgi apparatus and idiosome is the essential thing.

The facts thus far elucidated show clearly the basis of many of the older descriptions of the spermatid idiosome. The acetic acid in the fixative, or other technical failings, caused the Golgi apparatus to be overlooked entirely during the spermatocyte divisions. The idiosome, once reconstituted in the spermatid, became visible again with the older methods, and was called an idiosome because it looked like its predecessor

Fig. 2 Diagram to illustrate the history of the idiosome and Golgi apparatus in the differentiation of the sperm. The idiosome and the mitochondria (*nebenkern*) are stippled; the centrioles and tail filaments are indicated, together with the outlines of nucleus and cytosome. *A*, acrosome; *a*, acroblast; *N*, *nebenkern*. *A*, *B*, *C*, typical early spermatid (Hemiptera; Bowen, '20), showing idiosomic conditions directly comparable to those of figures 1*A*, *C*, *D*; *D*, differentiation of the vesicular type of acrosome (Hemiptera; Bowen, '20); *E*, differentiation of the granular type of acrosome (Mollusca; Schitz, '16); *F*, deposition of the migratory type of vesicular acrosome (Hemiptera; Bowen, '20). The arrows indicate the direction of subsequent movement of the acrosome and acroblast (Golgi remnant); *G*, deposition of the stationary type of vesicular acrosome (*Ceuthophilus*; Bowen, '22); *I*, deposition of the stationary type of granular acrosome (Mollusca; Schitz, '16). In figures *G*, and *I* the arrow indicates the direction of subsequent movement of the acroblast.



in the spermatocytes of the same or some other animal. What was once a mere guess is now known to have the best of basis in fact. Furthermore, the appearance of this body near the nebenkern in insects was the obvious source of the long confusion which resulted from tracing the idiosome to spindle remnants, mitochondrial derivatives, and every other possible origin. The acrosome of the mature sperm is to be derived from the complex formed thus by the idiosome and Golgi apparatus, which I shall accordingly refer to henceforth as the *acroblast*. The *acroblast* is the exact equivalent of the idiosome plus Golgi apparatus of the spermatocytes.

The origin of the acrosome from the *acroblast* is essentially similar in all animals belonging to Type I, but the nature of the acrosome itself makes possible a subdivision on morphological grounds, as follows: Type I, vesicular; type II, granular.

The *vesicular* type of acrosome is of very common occurrence, appearing in typical form in many Mammalia, Hemiptera, and Amphibia. In this case the acrosome makes its first appearance as a small, clear, bubble-like vesicle⁶ either within the idiosomic substance of the *acroblast* or on that part of its periphery from which the Golgi material is lacking. This vesicle gradually enlarges and soon projects conspicuously on one side of the *acroblast* (fig. 2D). The relation of the vesicle to the nucleus varies greatly in different forms, but the acrosome-*acroblast* complex seems in general to occupy some position near the spermatid nucleus. In the Hemiptera (fig. 2D) the vesicle is always in contact with the nuclear membrane; in *Locusta* (Otte, '07) the *acroblast* may be in contact with the nuclear membrane, while in *Ceuthophilus* (Bowen, '22) no particular relation seems regularly to occur. The ultimate size of the vesicle in proportion to the *acroblast* varies greatly in different animals, but the meaning of these differences is not known. Within the vesicle a darkly staining granule is characteristically developed, the granule being located typically on the inner wall

⁶ In some animals several such vesicles may be produced simultaneously, the separate rudiments thus developed being subsequently merged to form a single vesicle.

of the vesicle at the point where it is in contact with the nucleus (fig. 2D). For convenience in description, I propose to call the vesicle, the *acrosomal vesicle*, and the granule, the *acrosomal granule*; together they form the *acrosome*.

The *granular* type of acrosome is of much less frequent occurrence than the vesicular type, having been worked out with any completeness only in Mollusca (see, for example, Schitz, '16, on *Columbella* and Gatenby, '19, on *Paludina*). In this type there is developed on the periphery of the acroblast a small, darkly staining granule, instead of the clear vesicle characteristic of the preceding case (fig. 2E). No definite relation between the nuclear wall and the acrosome seems to exist.

The homologies which presumably exist between the vesicular and granular types of acrosome are not at present evident, for we do not know whether the granular acrosome is to be compared with the vesicular acrosome as a whole, or only with the acrosomal granule which is developed within it. It is of course possible that the granular type may be produced by faulty differentiation, and that its fundamental similarity to the vesicular type is obscured by the stain. Furthermore, we do not understand in either case the exact source of the acrosome. Apparently it is produced in both cases by a differentiation of the acroblast material, but whether this involves both idiosome and Golgi apparatus, or is confined to the idiosome alone is not known. The important point to note is that the acrosome is a secondary product of the acroblast and that neither idiosomic nor Golgi material goes directly into its formation. After the acrosome is completely formed, the acroblast is separated from it (figs. 2F to I), gradually moves back through the cytoplasm of the sperm tail, and, after undergoing degenerative changes, is probably eventually cast out of the sperm in all cases together with other débris of spermiogenesis. After the separation of the acroblast from the acrosome, I have suggested (Bowen, '20) for it the name *Golgi remnant*, as a term of convenient description. The Golgi apparatus and idiosome are thus lost from the completed sperm, in which they are repre-

sented only by their differentiation product, the acrosome. Weigl ('12), and Gatenby and Woodger ('21), however, claim that in some mammals at least, a part of the Golgi material is left behind as a permanent contribution to the middle-piece of the sperm; but the exact status of this material is, I think, at present a matter of reasonable doubt.

The ultimate rôle of the acrosome is, in any case, to act as an apical piece for the sperm head. It must, in other words, be applied eventually to the anterior surface of the nucleus, this final location being attained, as a rule, not later than the earliest stages in the elongation of the nucleus (if any occurs) to form the sperm head. The method of deposition varies according to the location of the acrosome at the time when the acroblast is separated from it. On this basis, two types of acrosomal deposition can be recognized, as follows: Type I, migratory; type II, stationary.

In the *migratory* type the acrosome is developed at some point removed by a greater or less distance from the apical pole of the nucleus, and the acroblast is cast off with the acrosome still at some distance from its final place of fixation (fig. 2*F*). In this case the completed acrosome is left resting at some point on the nuclear wall, whence it migrates (as indicated by the arrow in fig. 2*F*) around the nucleus to its definitive position. This migratory type is especially characteristic of the Hemiptera (Bowen, '20), in which the vesicular type of acrosome occurs. I do not know of any examples of this migratory type in the granular type of acrosome.

In the *stationary* type, the acroblast and acrosome move together to the anterior surface of the nucleus. Here the acrosome is deposited in situ (figs. 2*G* and *I*), and the acroblast (Golgi remnant) is then gradually separated from it, behaving subsequently exactly as in the migratory type. Examples of the stationary type among vesicular acrosomes (fig. 2*G*) are quite common, those of the mammal (see, for example, Gatenby and Woodger, '21), *Ceuthophilus* (Bowen, '22), and probably the salamander (Bowen, '22) being representative examples. The molluscs present many examples of the stationary, granular type (fig. 2*I*) of acrosome (cf. Schitz, '16, and Gatenby, '19).

The exact fate of the acrosomal granule in the vesicular type is in most cases unknown, though it seems, in Hemiptera for example, to be related to a darkly staining part which forms the tip of the acrosome. Acrosomes, particularly of the vesicular type, may undergo a great variety of differentiations, the case of *Locusta* (Otte, '07) being an especially good example; but the various shapes and structural features are not known to possess any points of general interest.

In figure 2 I have endeavored to represent in partially diagrammatic form the various types of behavior which occur in the simple or fused method of acrosomal formation. Beginning with a common type of acroblast (figs. 2A to C), either of two lines of development may be followed, leading to the production of an acrosome of either the vesicular or granular type (figs. 2D and E). The acrosome completed, it may either be deposited in situ or migrate to its definitive location subsequent to its deposition (figs. 2F, G and I).

Type II, Compound or multiple

The compound or multiple method of forming the acrosome is very incompletely known and seems, furthermore, to be of rather infrequent occurrence. At any rate, it has been observed very rarely, though this fact may be due to the much greater difficulties involved in its proper demonstration. In this type the Golgi bodies in the spermatid undergo little or no fusion (fig. 3), so that a single acroblast such as is figured in figures 2A to C is never produced. Instead, each of the Golgi bodies is apparently a small acroblast in itself—a probable interpretation which is borne out by the fact that in the fused type the acroblast is occasionally bipartite, each partial acroblast forming an acrosome proportional in size to the available material. The deposition of the partial acrosomes from these multiple acroblasts results presumably in the gradual building up of the complete acrosome. This process could undoubtedly be carefully analyzed in an animal which produced a vesicular type of acrosome from multiple acroblasts of sufficient size, and my observations on the Lepidoptera suggest that some of the moths may furnish material suitable for the purpose.

Thus far only two cases of multiple acroblasts are known, viz., the grasshopper (Bowen, '22) and the moths and butterflies.⁷ In the grasshopper the acrosome is apparently of the granular type (fig. 3), but all the parts concerned are so minute that thus far the exact details of the formation of the acrosome have not been made out. The granular acrosome is very small and is formed near the base of the head, moving thence around the nucleus to its apical side just prior to the drawing out of

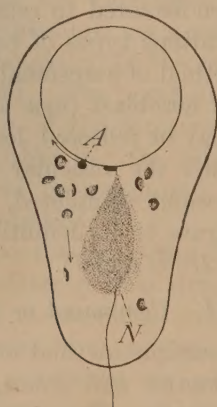


Fig. 3. Diagram to illustrate the formation of the acrosome from the multiple type of acroblast (acrididae; Bowen, '22). The arrows indicate the direction of the later movements of acrosome and acroblast. A, acrosome; N, nebenkern (mitochondria).

the sperm head. It thus belongs without question to the migratory type. In the *Lepidoptera* the acrosome is of the vesicular type, and is sometimes very large. In *Pygaera* the acrosomal granule itself is of extraordinary size and prominence. Here again the acrosome seems to be of the migratory type, but again the exact relation of the Golgi bodies to the parts of the acrosome has not been conclusively demonstrated. However, the multiple origin of the acrosome is clear, and the connection of the Golgi bodies with its formation is likewise beyond doubt. Thus far all my observations tend to bear out the

⁷ The notes here given on the *Lepidoptera* are largely based on a study of *Pygaera* and *Callosamia*, the results of which I have not yet published.

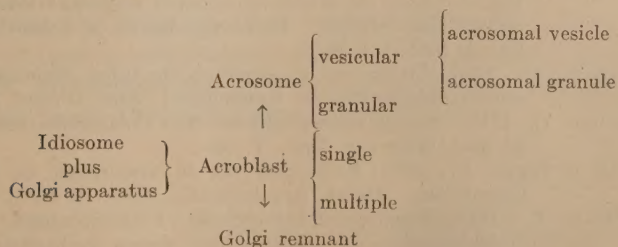
accuracy of the interpretation of the multiple type of acroblast which has been outlined above.

Aside from the single and multiple types of acroblast, an intermediate condition is an obvious possibility, but I am not acquainted with any form in which the facts are actually indicative of such a condition.

In the following table I have classified some of the better-known examples of acrosome formation according to the types which have been outlined in this paper.

GROUP	TYPE OF ACROBLAST	KIND OF ACROSOME	PLACE OF DEPOSITION
<i>Insecta</i>			
Hemiptera	Single	Vesicular	Migratory
Coleoptera	Single	Vesicular	?
Orthoptera			
Acrididae	Multiple	Granular	Migratory
Tettigoniidae	Single	Vesicular	Stationary
Lepidoptera	Multiple	Vesicular	Migratory
<i>Mollusca</i>	Single	Granular	Stationary
<i>Amphibia</i>			
Urodelia	Single	Vesicular	Stationary (?)
<i>Mammalia</i>	Single	Vesicular	Stationary

The nomenclature employed in this paper with reference to the spermatid structures may be outlined as follows:



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